

# ANNALS OF BOTANY

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## Studies in the Morphology and Anatomy of the Ophioglossaceae.

### III. On the Anatomy and Branching of the Rhizome of *Helminthostachys zeylanica*.

BY

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With Plates I-III and eight Figures in the Text.

IN the first of these studies<sup>1</sup> the morphology and anatomy of the rhizome of *Botrychium lunaria* was re-examined, and the anatomical relations of branches to the main axis was described. The corresponding facts for *Helminthostachys* will be dealt with in this paper. Our knowledge of this plant is fairly full and complete, but only the previous work on the rhizome and the anatomy of the stele and leaf-trace need be referred to here. Our chief source of information on these points is the account given by Farmer and Freeman<sup>2</sup>. In their paper the general morphology of the rhizome, its stelar structure, and the nature of the vascular supply to the roots and leaves are all described and figured. Reference is also made to the occurrence of branches, arising as 'adventitious buds' on older portions of the rhizome, and often springing from decorticated fragments, but no details of the branching<sup>3</sup> are given. To the description of the rhizome given by these investigators, Gwynne-Vaughan<sup>4</sup> added the recognition and description of vestigial or rudimentary axillary buds, one of which is constantly present in relation to each leaf. The anatomy of young plants attached to prothalli was briefly described by myself<sup>5</sup> and has been further dealt with by Campbell.<sup>6</sup> Some features of the leaf-trace have been described by Bertrand and Cornaille<sup>7</sup> and by Sinnott.<sup>8</sup> Preliminary accounts of some of the facts to be described below have been published in the Proceedings of the Manchester Literary and Philosophical Society.<sup>9</sup>

<sup>1</sup> Ann. of Bot., xxvii (1913), p. 203.

<sup>2</sup> Ann. of Bot., xiii (1899), p. 421.

<sup>3</sup> Ann. of Bot., xvi (1902), p. 17c.

<sup>4</sup> Ann. of Bot., xvi (1902), p. 41.

<sup>5</sup> The Eusporangiateae (Washington, 1911).

<sup>6</sup> Travaux et Mémoires de l'Université de Lille, tome x, mém. 29.

<sup>7</sup> Ann. of Bot., xxv (1911), p. 168, Pl. XI, Fig. 13.

<sup>8</sup> Vol. lvi, Pt. 2 (1912).



While this general reference to previous investigations on the rhizome of *Helminthostachys* will be sufficient, the main structural features, which are matters of common knowledge, may be briefly summarized. The horizontal, subterranean rhizome is definitely dorsiventral. The leaves, alternating with one another, arise in two dorso-lateral rows, while roots spring from the lower side and the flanks of the rhizome. The basal region of each leaf forms a peculiar sheath, which covers over the next younger leaf, and is broken through on the expansion of the latter. The narrow canal leading to the deeply seated vestigial bud continues backwards and inwards from the axil of the sheathing leaf-base. The rhizome has a wide parenchymatous cortex. The stele is tubular, but is not a solenostele, since there is no trace of internal phloem. It is bounded by the outer endodermis, within which come in order pericycle, phloem, conjunctive parenchyma, xylem, and pith. An internal endodermis abutting closely on the inner surface of the xylem is usually present in large rhizomes; it may be more or less incomplete, or wanting.

The cells of the pith resemble the cortical cells, and like them are often packed with starch. Cells with peculiar contents, recognized by Campbell as corresponding to the tannin cells of the Marattiaceae, may occur in pith and cortex. The xylem in adult medullated rhizomes is characteristically mesarch. Both outer (centrifugal) and inner (centripetal) xylems are well represented, their distinction being easy, as the mesarch protoxylem is often well marked. The vascular strands of the roots are attached to the sides or lower face of the stelar tube, while the upper side of the latter is disturbed by the departure of the alternating, mesarch leaf-traces. Each trace leaves a long narrow gap in the stelar tube; this usually closes just before the next leaf-gap is formed. A further complexity on the dorsal side of the tube results from a disturbance of the xylem in relation to each vestigial axillary bud; this is found, as a rule, just as the corresponding leaf-gap closes. The rhizome of the young plant shows a similar disposition of leaves and roots. Owing, however, to its more slender configuration and smaller stele, the leaves, though really in two rows, appear to spring in a single dorsal line. The stele may have a solid cylinder of xylem, or may, almost from the first, show a small pith. No definite leaf-gaps are left on the departure of the early leaf-traces, but a vestigial bud occupies the usual axillary position in relation to each leaf-trace. The transition from the slender rhizome of the young plant to the full size and usual structure appears to be a gradual one.

This summary of familiar facts will be sufficient introduction to the more critical consideration of some particular features of the rhizome of *Helminthostachys*. The difficult questions relating to the apical development and its relation to the segmental arrangement, indicated in the succession of the leaves and at least suggested by that of the roots, will

not be dealt with here. This paper will be limited to the anatomy of the developed regions of young and adult rhizomes, and the facts will be conveniently described in the following order:

1. Some points in the anatomy of the adult rhizomes. These include the relative development of centripetal and centrifugal xylem, the vascular attachment of the roots, the origin of the leaf-trace and its structure until after the first division in the cortex, variants in type of leaf-trace, and the disturbance of the vascular structure in relation to the vestigial buds.

2. The anatomical relations of actual branches to the main rhizome, and the associated development of accessory or secondary xylem.

3. The structure of the stele and leaf-trace in small rhizomes with the juvenile type of structure, whether arising as branches, or as young plants developed from an embryo; the progression from the juvenile to the adult type of structure, and condensation of stelar structure from the adult to the juvenile type.

The distinction made above, between the adult and juvenile types of rhizome and stele, is of practical convenience, but cannot of course be definite. Rhizomes, the stele of which has a pith and a clear distinction of outer and inner metaxylem, both consisting of pitted tracheides, will be classed as adult; those in which the stele is solid, or if medullated has no definite inner metaxylem composed of pitted tracheides, as juvenile. The transition from the juvenile to the adult type of stele is associated with an increase in the diameter of the rhizome, but the adult type is found in rhizomes of a wide range in thickness.

I am greatly indebted to Prof. F. W. Oliver and Prof. Bower for interesting pieces of rhizome of *Helminthostachys*.

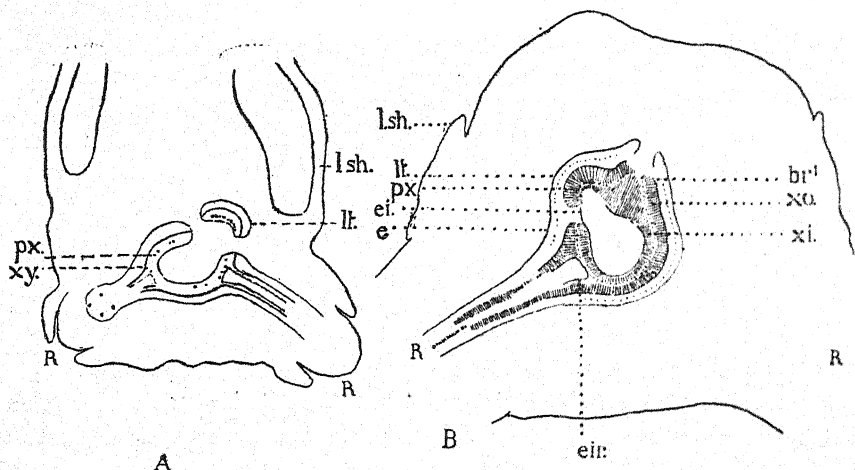
#### I. SOME POINTS IN THE ANATOMY OF THE ADULT RHIZOME.

The general features of a transverse section of a large rhizome of *Helminthostachys* are illustrated in Text-fig. 1, B. This shows the proportional sizes of the rhizome and stele, and the distinction between the dorsal side of the rhizome, where the remains of the attachment of an old leaf-sheath are seen, and the ventral side, where two large laterally placed roots are attached. The section has passed through the stele, where the xylem of a nascent leaf-trace has just become detached from the stelar tube on the side away from the median line. On the other side of the median line is seen the vascular disturbance, in relation to the vestigial bud belonging to the leaf next behind. The stele is surrounded by the external endodermis, which is only interrupted in relation to the vestigial bud, while the pith is delimited by a well-marked endodermis.

The convention adopted in this figure for representing the position and extent of the various tissues is maintained in many of the other text-figures, and may be explained once for all. The external endodermis is represented by

a continuous line, the phloem by a dotted line internal to this. The outer and inner metaxylems are represented by two sets of radially directed lines; between them the protoxylem elements occur in groups, and are represented in black in relation to the leaf-trace, but are usually not specially indicated in the rest of the stele. Though diagrammatic in the representation of the tissues, these figures are based on camera-lucida drawings, and represent the position and extent of the various tissues as accurately as possible.

It will be evident that the inner metaxylem is least strongly developed at the ventral side of the stele. The greater thickness of the xylem-tube



TEXT-FIG. 1. A. Section across adult rhizome near to the growing region, showing the attachment of the roots and their relation to the cortex. Only the outline of the stele and the outer limit of the xylem are indicated. B. Similar section through mature region of the rhizome. The stele of the rhizome shows a departing leaf-trace to the left of the median dorsal line, and the disturbance in relation to a vestigial bud on the left. There is an internal endodermis in the base of the root, unconnected with that in the rhizome. *lsh.*, leaf-sheath; *R*, roots; *xy.*, xylem; *px.*, protoxylem; *xo.*, outer xylem; *xi.*, inner xylem; *lt.*, leaf-trace; *br.*, vascular disturbance for vestigial bud; *e.*, outer endodermis; *e.i.*, internal endodermis of rhizome; *e.i.r.*, internal endodermis of root.

dorsally is mainly, though not entirely, due to the greater development of inner xylem there, especially in relation to the vestigial bud.

*Proportions of inner and outer metaxylem.* In rhizomes of the adult type both xylems are always represented, but the degree of development of the inner xylem exhibits a wide range of variation. In large rhizomes, such as that just described, the inner xylem is well developed; a portion of the vascular tube of this type is shown in Pl. I, Photo 1. The tracheides of the outer xylem often show some indication of radial seriation, but this is to be related to the sequence of divisions in the procambial stage, and does not indicate a secondary development. The xylem in all normal rhizomes examined is wholly primary, though an anomalous secondary development will be described below in relation to the branching of the rhizome. The inner xylem is usually much less marked in smaller

rhizomes, and often does not form a continuous zone; a portion of a vascular tube of this type is shown in Pl. I, Photo 2. In such rhizomes the parenchyma of the pith may at places abut directly on the protoxylem (Pl. I, Photo 3). Such steles present an appearance in transverse section not unlike some steles of *Botrychium lunaria*,<sup>1</sup> in that the outline of the pith is rendered irregular by the scattered elements of centripetal xylem. The analysis of the primary xylem into outer and inner is, however, much easier in the case of *Helminthostachys*, since protoxylem elements are usually present at intervals in the tube of xylem, and are not confined to the region of a departing leaf-trace.

It is not necessary to enter into the histology of the xylem further than to say that, in longitudinal section, the tracheides of the inner and outer metaxylem are similar, with narrow and often multiseriate pits. The protoxylem elements are narrower and stain less deeply; they show spiral marking passing into a reticulum, in the meshes of which bordered pits appear.

*The vascular attachment of the roots.* The roots are developed close to the growing point of the rhizome, and the early growth of the root thus coincides with the cortex of the rhizome attaining its full thickness. In the light of this we can understand how it is that only a comparatively thin zone of the cortex is actually broken through by the emerging root; this zone corresponds in thickness to the preceding leaf-sheath. The root is thus borne on a pedicel, the inner portion of the root-stele having no cortex of its own distinct from that of the rhizome. Since the superficial layer of cortex which is broken through often wears off later, the roots on mature portions of the rhizome may appear as if exogenous. The general relations will be clear without further description from Text-fig. 1, A and B. These figures represent transverse sections close to the tip of a rhizome (A), and through a mature region (B), in both cases passing through the attachment of roots.

As Farmer and Freeman state, the pith of the base of the root may be indirectly continuous with the pith of the rhizome by means of the xylem parenchyma. This statement may be somewhat amplified. The root, especially at its base, has a wide pith, the cells of which resemble histologically those of the pith of the stem. The xylem of the root is continuous with the outer xylem of the stem-stele, and the xylem parenchyma is well marked beneath the base of the root. The pith of the root is continuous with this parenchyma, the position of which is usually between the inner and outer xylems of the rhizome, though sometimes a thin zone of outer xylem persists across the base of the root. When the inner xylem is well developed, it separates the pith of the root from that of the rhizome (cf. Text-figs. 1, B, 3, B, C, and Pl. I, Photo 4). When, however, the inner xylem

<sup>1</sup> Cf. Ann. of Bot., xxvi, Pl. XX, Photos 16, 17.

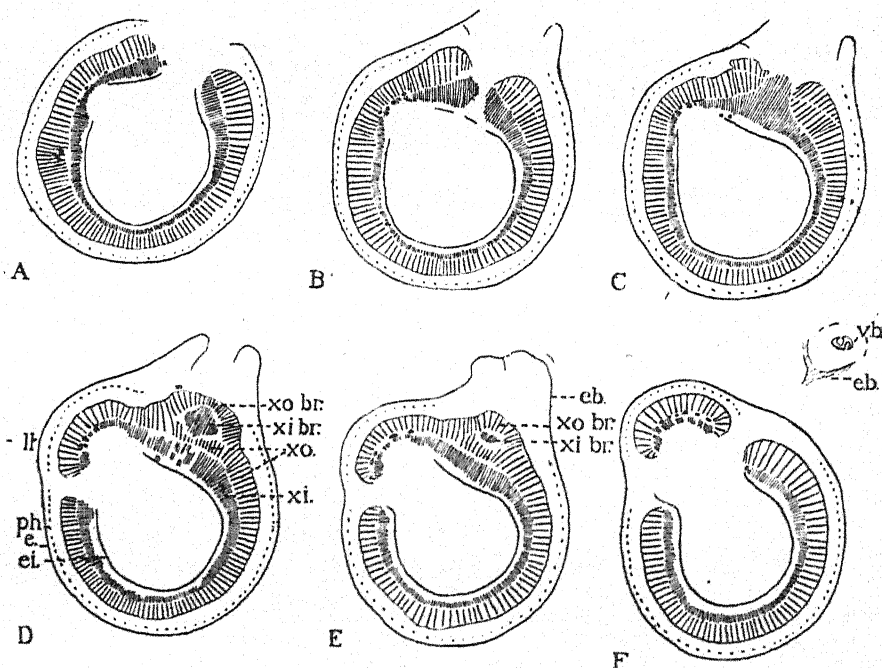
of the rhizome is less complete, the pith of the root appears continuous with that of the rhizome (cf. Pl. II, Photo 25). This occasional parenchymatous continuity between the pith of the rhizome and root is obviously of no significance as regards the nature or origin of the pith of the root.

It is of some interest to find that an internal endodermis may be developed in the basal region of a root, delimiting its pith. Such an endodermal layer may be more or less complete. Its position in a well-marked case is shown in Text-fig. 1, B. As in the case of the rhizome, the internal endodermis of the root may abut directly on the tracheides. There is clearly no reason to look on the internal endodermis of the root as anything but a new development, and its occurrence thus bears on the significance to be attached to the internal endodermis present in the rhizome.

*Origin and structure of the leaf-trace.* It has been pointed out above that the dorsal side of the stele is of special interest, since the leaf-traces depart from it, and also because of the vascular disturbance in relation to the vestigial buds (cf. Text-fig. 1, B). As is well known, the departing trace leaves a long narrow leaf-gap in the medullated stele. This gap closes before the next leaf-trace separates, so that the gaps do not usually overlap, and their mutual relation is similar to what is found in many dorsiventral solenostelic Ferns. The vestigial bud discovered by Gwynne-Vaughan is situated just in front of the closed leaf-gap to which it belongs, and the vascular disturbance in relation to the bud is found just as the gap closes. The vascular disturbance thus lies alongside the separating trace for the next leaf (Text-fig. 1, B). A series of sections through the region of separation of one leaf-trace will thus show the closure of the preceding leaf-gap on the other side of the median line, and the vascular relations of the vestigial bud. Such a series from a large rhizome is represented in Text-fig. 2; it shows to the left the earlier stages in the separation of a leaf-trace, and on the right the closure of the preceding leaf-gap, the development of the bulge of xylem in relation to the vestigial bud, and the return to the normal thickness of the vascular tube in front of this. Leaving the consideration of the vestigial bud to the next section, the origin, separation, and further history of the leaf-trace in this specimen may now be considered.

This rhizome had a complete internal endodermis, and the inner metaxylem was well developed. The first indication of the position and extent of the leaf-trace was afforded by the disappearance of the endodermal characters from the cells internal to the nascent trace. The xylem tube in the region of the trace also appeared thinner, owing to a less development of centripetal xylem. This is the condition reached at the level of the section in Text-fig. 2, A; the adjoining leaf-gap has begun to close, while the position of the nascent leaf-trace had been recognizable some distance behind the level of this section. The series of figures in Text-fig. 2 shows

the gradual arching out of the leaf-trace xylem (Text-fig. 2, A, B, C), its separation from the xylem-tube first on the side away from the middle line of the stele (D, E), and shortly afterwards on the median side also. At this level (Text-fig. 2, F) the trace is distinct from the stele as regards its xylem and phloem, but is still enclosed by the unbroken stelar endodermis. On the other side of the middle line the previous leaf-gap is seen to have closed, the disturbance of the xylem to have subsided, and the



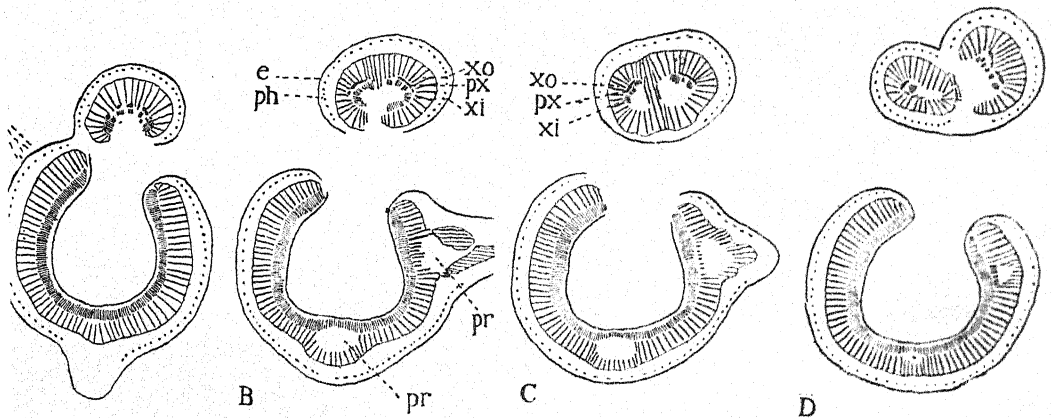
TEXT-FIG. 2. Series of transverse sections of the stele of a large rhizome, to show the early stages of departure of a leaf-trace and the closure of the leaf-gap of the preceding leaf and the development of a well-marked vestigial branch stele. Further description in text. *lt.*, leaf-trace; *ph.*, phloem; *e.*, outer endodermis; *e.i.*, internal endodermis; *x.o.*, outer xylem; *x.i.*, inner xylem; *x.o.br.*, outer xylem of vestigial branch; *x.i.br.*, inner xylem of vestigial branch; *v.b.*, inner end of canal to vestigial bud; *e.b.*, endodermis in relation to vestigial bud.

section passes through the inner end of the canal leading down to the vestigial bud.

The leaf-trace itself as it separates from the stele is mesarch, although the inner xylem is much less developed than in the stele of the rhizome. The trace in this large rhizome thus agrees with that described and figured by Sinnott, but, as will be seen below, this is by no means the only type of trace found in *Helminthostachys*.

The further changes undergone by the trace of this rhizome as it passes obliquely through the cortex are illustrated in Text-fig. 3; this represents the preceding trace, and is therefore on the other side of the

middle line, but otherwise continues the series of changes shown in Text-fig. 2. In Text-fig. 3, A, the endodermis is seen interrupted on the side away from the middle line, though on the other side it is continuous from the leaf-trace to the stele. The external and internal endodermal layers have become continuous round the margin of the leaf-gap. The leaf-trace is distinctly mesarch and the protoxylem elements form an interrupted band between the two xylems. In Text-fig. 3, B, the trace is completely separate, and comparison with the earlier stage will show that the arc of xylem is becoming more curved and is tending to complete the tube of outer xylem on the adaxial side. The persistence of the centripetal xylem internal to the protoxylem enables a clear distinction to be drawn between true mesarchy of the trace and this adaxial completion of the xylem. The



TEXT-FIG. 3. Series of sections from the same rhizome as Text-fig. 2, to show the later stages in departure of a leaf-trace, the adaxial completion of the xylem, and the clepsydroid stage passed through before division. Further description in text. *e.*, endodermis of leaf-trace; *ph.*, phloem; *x.o.*, outer xylem; *px.*, protoxylem of leaf-trace; *xi.*, inner xylem; *pr.*, pith of base of root.

trace in Text-fig. 3, B, shows preparations for division in that the protoxylem is present as two laterally placed groups; a projection of metaxylem between these is also to be noted. In the next stage figured (Text-fig. 3, C) the outer xylem has become complete adaxially and so has the endodermis; the phloem was not completed in this trace. The projection of xylem noted in the preceding stage has become continuous with the adaxial xylem, and divides the parenchyma in the concavity of the xylem into two areas. In each of these, internal to the protoxylem, centripetal xylem still occurs, though it is more poorly represented than lower down. Shortly after this, when the trace is approaching the outer part of the cortex, it divides into two. A stage of the division is represented in Text-fig. 3, D, and it will be noted that in this case the outer xylem forms a complete ring in one half of the trace and not in the other. The inner xylem is still



represented, but soon ceases to be recognizable in the bundles of the base of the leaf.

The stage represented in Text-fig. 3, C, is evidently that recognized by Bertrand and Cornaille<sup>1</sup> as an 'état très particulier de la trace clepsydroidé', but not described or figured in full. They describe it as 'une chaîne binaire, celle-ci est doublement fermée, étant composée de deux divergeants fermés'. The name given by these investigators to this stage will be adopted, and the significance of this clepsydroid stage will be discussed later. As a rule it is passed through rapidly, the complete division of the trace being effected in the outer part of the cortex.

The actual appearance of the large trace, the structure of which has been represented in Text-figs. 2 and 3, is shown in Pl. II, Photos 20, 21, and Pl. I, Photos 5 and 6. In Photo 21 the nascent trace is seen to the left, still forming part of the stelar tube. Photo 5 shows the adaxial completion of the xylem of the separated trace almost effected, while Photo 6 shows the trace in the clepsydroid stage. The low-power photograph in Pl. I, Photo 4 shows the trace in the clepsydroid stage in relation to the stele, from which it had departed.

This large trace, derived from a full-sized adult stele, has been described at some length, since it allows the distinction to be clearly made between the *mesarch condition*, i. e. the existence of outer and inner xylem corresponding to the outer and inner xylem in the stele of the rhizome, and the essentially different condition brought about by the *adaxial completion of the outer xylem* in the leaf-trace. Since the mesarch condition persisted until after this trace divided, the adaxial completion of the arc of outer (centrifugal) xylem could be seen to be quite distinct from the original mesarchy. The distinction of these two constructions, both resulting in the presence of metaxylem to the inside as well as the outside of the proto-xylem, must be borne in mind in considering other examples, the interpretation of which is less obvious.

Different pieces of rhizome, while agreeing in the general type of construction of stele and leaf-trace, exhibited variants of the type, which were usually maintained throughout the particular piece of rhizome. These variants are of special interest in the leaf-trace and concern its mode of departure, its construction in the cortex, and its mode of division. Some examples will now be briefly described and illustrated in order to show the nature of the range.

The division of the leaf-trace often takes place without the adaxial completion of the xylem or endodermis having been effected. This is found both in the case of leaf-traces that are mesarch at their departure and in the case of smaller traces that have no centripetal xylem. An example, though not an extreme one, of a dividing trace, in which the

<sup>1</sup> Loc. cit., p. 179, foot-note.



adaxial extension of xylem was incomplete at division, is given in Pl. I, Photos 7-9. In such cases no definite clepsydroid stage is passed through.

Even in the large mesarch traces described above, the inner xylem was found to diminish in the region of the nascent leaf-trace (cf. Pl. II, Photo 21). As a rule, in smaller rhizomes, where the centripetal xylem is less strongly developed, the inner xylem completely disappears from the middle of the concavity of the nascent trace, so that the parenchymatous tissue is continuous from the pith to the protoxylem. The trace thus separates from the stele with xylem, which is either completely endarch or has only traces of inner xylem to the two sides. Thus there is very little inner xylem in the trace figured in Pl. I, Photos 7-9, while Photo 10 shows a purely endarch trace which had just departed from another rhizome. This latter trace divided without any indication of adaxial completion of xylem. On the other hand, the trace shown in Pl. III, Photos 47-49 was endarch at its departure, but almost at once showed adaxial completion of its xylem.

The last-mentioned specimen thus illustrates another variant in which the adaxial completion of the outer xylem of the trace takes place while the latter is still monarch (Photo 48). This was followed later by the assumption of the clepsydroid structure preceding the division of the trace (Photo 49), which took place in the outer region of the cortex.

This pre-clepsydroid condition, in which the trace is adaxially completed, but has an undivided protoxylem, may be maintained until the trace has left the cortex of the rhizome. This was the case throughout a large fragment of rhizome which bore a branch, to be described later, and the progress outwards of one trace can be followed in Text-fig. 6 from the right side of the rhizome. This trace as it left the cortex is also shown in Pl. I, Photo 11, while Photos 12 and 13 on the same plate show another trace from the same rhizome, just before the xylem was completed adaxially and after this had been effected. There is no room for doubt as to the adaxial completion of the xylem in these traces, and the reality of the process is confirmed by the accompanying completion of the phloem, which is seen in Photo 13; and this is even better shown by the next rhizome to be described.

This piece of rhizome will be referred to later in the paper as showing a rapid progression from the juvenile to the adult type of structure. The type of leaf-trace departure, which was maintained at each node, was remarkable in that the xylem of the trace was completed adaxially before or as it separated from that of the stele. As the xylem of the trace enclosed little parenchyma, it was possible on the one hand that its adaxial portion represented the very early completion of the outer xylem of the trace, or, alternatively, that it was the continuation outwards of the inner xylem of the mesarch stele. Direct observation supports the former

explanation, and this interpretation seems justified in the light of comparison with the other types of trace described above.

The main stages in the departure of one of the earlier and smaller traces from this rhizome are represented in Pl. I, Photos 14-17. The rhizome at this level was medullated and mesarch, though with the internal xylem only moderately developed. As the xylem of the trace becomes evident as a bulge on the stele (Photo 14), the protoxylem has an arc of metaxylem to the outside, and this appears almost completed on the adaxial side of the trace, which is still continuous with the xylem of the stele. Thus as the xylem of the trace becomes separate (Photo 15), it exhibits a complete ring of radiating rows of tracheides, the single group of protoxylem being in connexion with the abaxial portion of the ring, while a little parenchyma is present immediately internal to the protoxylem. The adaxial xylem at this level appears to be related to the outer xylem of the stele, and is in any case much more strongly developed than the inner xylem of the stele. At this stage the stele and leaf-trace are enclosed in a common endodermis and the phloem is still continuous. Photo 16 shows the same trace further removed from the stele. It still has a single group of protoxylem, the xylem being almost equally developed abaxially and adaxially. The interpretation given of the adaxial completion of the xylem in this trace is confirmed by the corresponding behaviour of the phloem, which is seen to form a complete tube, while the endodermis was also complete. A little further out (Photo 17) the small trace had passed into the clepsydroid stage; the endodermis and phloem were still complete. The xylem appeared dumb-bell shaped in cross-section, the protoxylem had divided into two groups, and the metaxylem elements were continuous across the constricted portion between the two halves. Shortly after this the division of the trace took place; the xylem of each half was a complete ring, and the phloem was complete around it.

The departure of the larger leaf-traces from the full-sized portion of this rhizome was essentially similar, though the trace may be mesarch at its origin, and thus have some inner xylem enclosed by the adaxial extension of the outer xylem. One of the larger traces in the clepsydroid stage is represented in Pl. I, Photo 18. While corresponding essentially to the large trace shown in Photo 6, this trace, which is at a somewhat earlier stage, has no evident remains of inner xylem. The two poles of protoxylem are clearly evident, and the adaxial arc of xylem is actually thicker than the abaxial portion. A little later the metaxylem develops in the centre of the trace connecting the abaxial and adaxial portions, and the trace then divides (cf. Text-fig. 8, E). In Pl. I, Photo 19 the left half of this trace is shown with its protoxylem in two groups preparatory to the next division.

The trace shown departing from the moderately small rhizome in Pl. III, Photo 51 agreed with those just described in having the xylem of

the trace completed adaxially before it had separated from the stelar xylem. In this case there was no difficulty in the interpretation, since the inner xylem first disappeared from within the nascent trace. In the trace figured the adaxial xylem is almost complete, and is well represented on the left side.

The variety shown by the leaf-trace in different pieces of rhizome of *Helminthostachys* is remarkable, and has necessitated the description of a number of examples. A general plan will, however, be seen to underlie the variety. The xylem of the trace is ultimately equivalent to, and derived from, an arc of the outer xylem of the stelar tube. No inner xylem may be continuous into the trace, or the latter may be mesarch at its base, the inner xylem dying out sooner or later. The outer xylem tends to be completed adaxially (though this does not always take place); the resulting construction is essentially distinct from mesarchy as we find it in the stelar tube of the xylem. In preparation for division the protoxylem separates into two groups, and the trace passes through a clepsydroid stage that is often clearly marked and striking. The monarch pre-clepsydroid condition may, however, persist until the trace leaves the rhizome. More usually the trace has divided twice before this takes place, the complex vascular system of the petiole being thus continued backwards into the cortex of the rhizome. In all cases observed, however, the trace is monarch as it separates from the stele, though the continuation backwards of the double condition seen in the clepsydroid stage would be a readily comprehensible term in the series of variants.

Parallel variants in the structure and relations of the leaf-traces will be referred to below in considering more slender rhizomes showing juvenile structure, and the whole question will be discussed at the end of the paper.

*The vascular disturbance in relation to the vestigial buds.* The original description by Gwynne-Vaughan<sup>1</sup> of the structures which must undoubtedly be regarded as vestigial or dormant lateral buds, or rather apices, was illustrated by clear diagrammatic figures. These show the course of the narrow canal extending backwards and inwards from the axil of a leaf-sheath to the neighbourhood of the stele just in front of the closed leaf-gap corresponding to the subtending leaf, and also the disturbance of the stele in relation to the vestigial structure. As his figure shows, the canal turns slightly forwards at its lower end, and, so far as I can judge, the dormant apex is situated behind this inner portion. As regards the structure of the vestigial bud itself, I can at present add little to Gwynne-Vaughan's account. It seems to consist only of a dormant apical meristem, the structure of which is difficult to make out in the absence of segmentation. It is of some interest, for comparison with the main apex, to find that the lower portion of the canal may be enlarged by the development of hairs projecting into it.

<sup>1</sup> Loc. cit., p. 171, Fig. 19.

As regards the vascular disturbance, Gwynne-Vaughan's figure indicates that the inner xylem first closes across the gap, and also that the increased thickness of the xylem leads to the existence of a distinct low bulge behind and below the vestigial bud itself.

The analysis of this bulge is not carried further by him, and it is of interest to ascertain the parts taken in its formation by the outer and inner xylems respectively. This was studied in the large rhizome, the departure of the leaf-trace from which has been already described, and the same series of figures (Text-fig. 2) will serve to illustrate the anatomical relations of a vestigial bud in a case in which the vascular disturbance was well marked.

After a leaf-trace has departed, the gap in the stele remains open for a considerable distance. On following a series of sections forward, the gap in the tube of xylem is seen to narrow, and its edges to thicken as preparations for closure become recognizable (Text-fig. 2, A, B). The thickening is largely due to an increase in the amount of inner xylem, which at this region becomes more strongly developed than elsewhere in the stele; there is also an increase in the thickness of the outer xylem, so that the wood bulges both outwards and inwards in the region of the closing gap (Text-fig. 2, B). The endodermis appears raised up over the margins of the gap. The closure of the gap comes about by the meeting of the edges formed by the bulges of inner xylem. This not only joins across the gap, but the inner xylem appears to extend outwards into the gap still present in the outer xylem (Text-fig. 2, C). A little further forward (Text-fig. 2, D) the outer xylem is completed over this extension of the inner xylem through the gap. Since the outer xylem also completes the xylem of the main stele, it separates the projecting portion of the inner xylem from the corresponding tissue of the main stele. At this level, therefore (Text-fig. 2, D), the vascular bulge can be regarded as a rudimentary branch stele, with its own inner and outer xylem. Still further forward the bulge of xylem is subsiding, and at this level the endodermis was completed over the parenchymatous bulge leading towards the vestigial bud (Text-fig. 2, E). The last section figured (Text-fig. 2, F) is immediately in front of the actual bud, and shows the inner portion of the canal occupied by hairs, and beneath this the forwardly bent portion of the endodermal bulge. The stele of the rhizome has resumed its normal structure, and the endodermis is complete around it.

The description of this vascular disturbance just given naturally involves a view as to the interpretation of the structure. This interpretation has been reached by comparison of a number of specimens, and will be seen below to be supported by the nature of the vascular supply to actual branches. The appearance of the vascular bulge in the specimen described is shown in Pl. II, Photos 20 and 21. Photo 20 shows the dorsal region of the stele just after the leaf-gap has been closed by the approximation of

the internal xylem, which can be traced bulging outwards into the gap. The level is therefore about the same as Text-fig. 2, C. The second photograph (Photo 21) shows the bulge with the outer xylem completed over the inner xylem and also extending across to complete the xylem tube of the rhizome. The level of this section corresponds to Text-fig. 2, D. The whole structure suggests the attachment of the base of a miniature branch stele, with its inner and outer xylem continuous with the corresponding tissues of the main stele.

The vascular disturbance in relation to the bud was exceptionally well marked in the large rhizome selected for description above, and in a number of similar pieces of rhizome (cf. Text-fig. 1, B). In other and smaller rhizomes, however, the structure was different, though such cases did not negative the interpretation of the better marked and more highly organized examples. In smaller rhizomes the closure of the leaf-gap took place by the meeting of the relatively strongly developed xylem at its edges; the inner xylem sometimes extended slightly outwards into the gap, the outer xylem not being continued across till later. But in these cases (cf. Pl. I, Photo 7) no well-marked vascular bulge was formed, and there was no indication of an organized branch stele, although the xylem ring was thicker in this region owing to the greater development of inner xylem. The endodermis was raised up and open in relation to the vestigial bud; sometimes it had previously closed around the stele, in other cases it remained open.

In small rhizomes also the bud often appears to be situated relatively further back. In the full-sized adult rhizome the transverse section showing the actual bud passes through the leaf-trace on the other side of the median line, when it has separated from the vascular tissues of the stele. This would correspond to a section between E and F in Text-fig. 2. In the small rhizomes, on the other hand, the next leaf-trace may still form part of a complete stelar tube at this level (cf. Pl. I, Photo 7), and in other cases the bud may be so far back as to lie over the unclosed leaf-gap (cf. Text-fig. 4, A). These differences in position do not affect the regular segmental succession of leaves and buds, but are of interest for comparison with what is found in rhizomes of juvenile type, and as bearing on the attachment of actual branches.

## 2. THE ANATOMICAL RELATIONS OF ACTUAL BRANCHES TO THE MAIN RHIZOME.

In Farmer and Freeman's paper<sup>1</sup> reference is made to the occurrence of 'adventitious' branches on more or less decorticated fragments of rhizome, but no account is given of the vascular relations of the branch to the main stem. Gwynne-Vaughan<sup>2</sup> suggested that such branches might be due to the vestigial buds being stimulated into action under certain

<sup>1</sup> Loc. cit., p. 423.

<sup>2</sup> Loc. cit., p. 173.

conditions. The following account is in complete accordance with Gwynne-Vaughan's interpretation of the morphology, the correctness of which was indeed evident from the corresponding facts since made out in *Botrychium*.

I obtained two specimens of *Helminthostachys*, each showing a small branch arising from a short piece of a main axis. The development of the branches thus appeared to be associated with arrest of the normal growth of the main axis. In this respect, as well as in the structure and position of the dormant buds, a close parallel with *Botrychium* can be traced.

The first branching specimen was a small piece of rhizome that to all appearance terminated in its apical bud. When cut into a complete series of sections, however, it proved to consist of axes of two orders. The base was a fragment of a small rhizome of adult type, including seven leaf-gaps; while borne on this, and arising from the dormant bud in relation to the most anterior leaf-gap, was a lateral branch. This had continued the line of growth of the arrested main axis. The specimen was thus particularly favourable for tracing the relations of the vascular system of the branch to that of the main stem in a series of transverse sections.

The structure of the small rhizome, which bore the branch, may be very briefly summarized (cf. Text-fig. 4, and Pl. II, Photos 22-24). The stele had a well-marked pith not limited by an internal endodermis. The xylem was mainly outer or centrifugal. In some regions the inner xylem was fairly developed, but for the greater part of the length of the fragment it was feebly represented by isolated tracheides or groups of tracheides. It was as usual best represented towards the upper side of the stele. It disappeared internal to the leaf-trace, so that the latter departed with an endarch strand of xylem (Pl. I, Photo 10). The trace passed rapidly through the cortex, dividing without adaxial closure of the xylem. A vestigial bud was present in relation to each leaf. Its general structure was as usual, but it may be noted that it was not so far in front of the subtending leaf-trace, but was situated over the leaf-gap before this had closed even in the xylem tube (Text-fig. 4, A, B). There was no definite vascular disturbance in relation to the bud. On passing forward, the vestigial bud over a leaf-gap is first met with (Text-fig. 4, A), the slit in relation to the bud then reaches the surface, and the leaf-gap closes (Text-fig. 4, B, C) before the separation of the next leaf-trace from the stelar tube commences. There is thus a region intervening between one leaf-gap and the next, in which the stele is tubular and undisturbed either by a vestigial bud or a departing leaf-trace. The appearance of a vestigial bud standing beside the separating trace for the next leaf is not seen. These differences in degree will be evident on comparing Text-figs. 2 and 4, and have to be borne in mind in considering the relations of the actual branch.

The departure of the leaf-trace subtending this branch and the vascular





supply to the branch can be followed from Text-fig. 4. The first section (A) shows the preceding leaf-gap still open and the position of the vestigial bud above it. On the other side of the median line the protoxylem of the next leaf-trace is recognizable, separated by inner xylem from the pith. In B the condition is similar, but the section now passes through the canal leading to the vestigial bud, and the gap in the xylem is narrowing. In C the canal has almost reached the surface of the rhizome, and the leaf-gap is closed, although the external xylem is not of full thickness across it. On the other side of the middle line, the nascent leaf-trace is more evident, and the inner xylem has largely disappeared opposite its protoxylem. To either side of the xylem of the trace, which still forms part of the complete stelar ring, the first indication of the vascular supply to the branch is visible. At these points an additional development of tracheides has taken place immediately outside those of the normal xylem. This xylem, which is indicated in the figures by cross-hatched shading, corresponds in a sense to secondary xylem, and will be referred to as *accessory xylem*. In the next section (D) these patches of accessory xylem are much larger, and the new development extends further round the stele, though confined to its upper side. The leaf-trace is seen to be purely endarch as it departs, and the inner xylem is wanting opposite to it. The separation of the trace is further advanced in E; this shows a new feature, in that the inner xylem, which at the preceding level was well marked to either side of the nascent leaf-gap, now extends across below the gap, and also out into it. This inner xylem, together with the accessory xylem evident to either side of the leaf-gap, is destined to supply the stele of the branch subtended by the departing leaf-trace. The actual appearance of the stele about this level is shown in Pl. II, Photo 22, in which the tracheides of the accessory xylem (*x.<sup>2</sup>*), and those of the inner xylem filling up the gap (*x.i.*) will be seen to have stained more faintly than those composing the normal primary xylem of stele and leaf-trace. The next sections in Text-fig. 4 show how the xylem of the branch stele becomes constituted. At the level of Text-fig. 4, F, the leaf-trace was departing from the cortex. The endodermis has become completed over the leaf-gap, and the inner xylem has passed outwards through the gap, and come into contact with the two groups of accessory outer xylem that have extended towards the middle line of the gap. The actual appearance about this level is shown in Pl. II, Photo 23. In G, the two groups of accessory xylem have joined to form an arc of xylem, against the inner face of which lies the group of inner xylem. The xylem of this nascent branch-stele is now distinctly separated by parenchyma from the main stele, the gap in the xylem of which is seen to be still open. The outer xylem of the branch stele now becomes continued around the inner xylem, between the elements of which parenchyma has appeared. This process, begun in Text-fig. 4, H, is complete in I, although



the zone of the outer xylem is thin on the adaxial side: in J the outer xylem is more uniform in thickness, and the stele of the branch may be regarded as fully constituted. The actual appearance at this level is shown in Pl. II, Photo 24. Meanwhile, as Text-fig. 4, H, I, J, shows, endodermal markings have appeared in a layer of cells between the branch stele and the main stele, and this new-formed layer of endodermis has become continuous with the endodermis on the abaxial side of the branch stele to form the complete endodermis of the latter. By this stage, when the completed branch stele lies in the cortex beside that of the main rhizome, the leaf-gap in the xylem of the latter has closed, and the position of the next leaf-trace is evident. The end of the fragment of rhizome is, however, almost reached, and the broken stele is in great part decorticated (Text-fig. 4, J; Pl. II, Photo 24).

It is thus evident that the outer and inner xylem of the branch stele are continuous with the outer and inner xylem of the main rhizome, and that the endodermis is similarly continuous from the main stem to the branch. The same holds almost certainly for the phloem, but on account of the difficulty of distinguishing the sieve-tubes, though they could be recognized at places in these transverse sections, no differentiation has been indicated in the zone between the endodermis and the xylem of the branch stele. This zone corresponds to pericycle, phloem, and conjunctive parenchyma. Such a branch stele agrees in essentials with the stele of a young plant developed from an embryo, and its further consideration will be deferred to the next section.

Two comparative points may be indicated in passing, although they will have to be discussed more fully later. Judging by the structure of the vascular elements, the stele of the branch would naturally be described as centrarch, i. e. as having protoxylem mixed with parenchyma centrally and the metaxylem around this. The connexions of these regions of the xylem of the branch stele have been clearly seen, however, to be respectively with the inner and outer metaxylems of the stem stele. There is thus reason for distinguishing inner and outer xylem in the smaller and simpler stele of the branch.

If the connexions of the inner and outer xylem of the branch stele (Text-fig. 4) be compared with the large vascular disturbance in relation to a vestigial bud that has already been described (Text-fig. 2), a remarkable agreement will be found. The bulge of xylem behind the vestigial bud was, like the stele of the branch, composed of (a) inner xylem passing out through the closing leaf-gap, and (b) outer xylem continuous with the thickened margins of the gap in the xylem. This agreement confirms the interpretation of the marked vascular disturbance suggested in the previous section of this paper.

The second branched specimen was an irregular, discoloured, and dying

fragment of a full-sized rhizome. It bore laterally a small shoot of pale colour, with healthy-looking tissues. The parent fragment had evidently been displaced in the soil, and the developing lateral branch had adjusted its direction of growth to the new position, and had grown out almost at right angles to the main rhizome (cf. Text-fig. 5). The interpretation of the anatomical relations between the main rhizome and branch was thus more complicated than in the first specimen. The altered direction of the branch stele had, however, the great advantage of giving a view of its basal region in longitudinal section, while the parent rhizome was studied in a complete series of transverse sections.

The fragment of rhizome had the fully adult type of structure, and included portions of four leaf-gaps; the first gap was open at the basal end, while the fourth gap was not closed when the fragment ended. Vestigial buds were present in relation to the first and third gaps, while the actual branch occupied the same position relatively to the second leaf-gap. The general structure of the stele resembled that of the large adult rhizome first described (cf. Pl. II, Photo 25). Inner xylem was present, though not so strongly developed, and no internal endodermis was recognized. The peculiarities of the leaf-trace have already been described (cf. Pl. I, Photos 11-13). The vascular changes accompanying the closure of the leaf-gap resembled on the whole those in the large rhizome described, and involved the formation of a large bulge of xylem behind the vestigial bud. The bud itself, i. e. the inner end of the canal, was on this rhizome situated relatively further forward, and the branch will be seen to stand beside a leaf-trace, the departure of which is far advanced. The main features of the rhizome are shown in Text-figs. 5 and 6.

In this case, as in the first branching specimen, the influence of the development of the branch extends backwards to the region of the stele, where the subtending leaf-trace is just becoming evident. This influence is expressed in the same way, namely, by the development of accessory xylem outside the ordinary outer primary metaxylem. In the preceding specimen this was only developed in such positions that it appeared to pass off entirely as the outer xylem of the branch stele. But in this larger rhizome the development of accessory xylem was not confined to the neighbourhood of the leaf-gap, but extended all round the stele. Further, the accessory xylem did not wholly pass off to the branch, but was present for a considerable distance after the departure of the branch. It thus presented an even more striking resemblance to secondary thickening than in the former case.

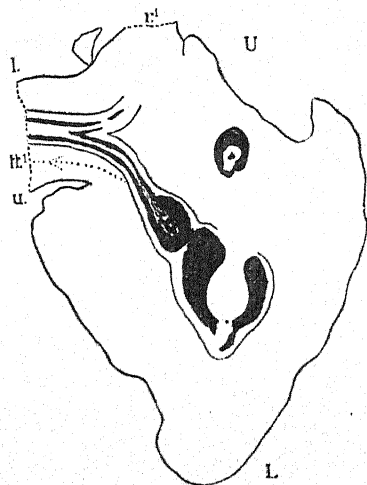
It will be convenient to describe the features of this accessory xylem more in detail before considering the origin of the branch. Its distribution will be evident from Text-fig. 6, in which it is indicated by cross-hatched shading. In the first section (A) of this figure, it is seen to be represented

all round the stele, although the branch stele is not yet reached, while in the last section (F) it is still present all round the stele, although the branch has passed off and the next leaf-trace has reached the surface of the rhizome. Its appearance in this region beyond the departure of the branch is shown in Pl. II, Photo 25, which is to the same scale, and may be compared with the ordinary primary structure seen in Pl. I, Photo 4. A portion of the stelar tube, showing the accessory xylem, is more highly magnified in Photo 26 on Pl. II. It will be sufficient to refer to this last figure, which should be compared with Pl. I, Photo 1, in the following description.

The endodermal markings were not pronounced in the necrosed tissues of this rhizome, but the approximate position of the endodermis is indicated at *e*. Within this comes the pericycle, and at *ph*. the phloem is clearly evident. Within this comes conjunctive parenchyma. In an ordinary rhizome the outer metaxylem (*x.o.*), would have come next followed by the inner metaxylem (*x.i.*) and the pith. The position of these tissues is indicated in Pl. II, Photo 26, but to the outside of the outer metaxylem the irregular band of accessory xylem (*x.<sup>2</sup>*) is present in addition. This has evidently been secondarily developed as the result of tangential divisions taking place in the cells of the conjunctive parenchyma adjoining the primary xylem, and cells showing tangential division-walls are evident in the photograph. Many of the resulting elements have developed into tracheides, but others have remained parenchymatous. It is due to this that the accessory xylem is at places continuous with the primary xylem, while at other places some parenchymatous secondary tissue intervenes (cf. Photo 25, Pl. II). This xylem was clearly a secondary development after the primary structure of the stele was completed. It occupies the usual position of a normal zone of secondary xylem relatively to the primary xylem and phloem. On the other hand it is irregular, in that no definite meristematic layer or cambium is formed or persists outside the newly formed xylem. Even allowing for these peculiarities, it seems to me clear that the development of this accessory xylem should rightly come under the conception of secondary thickening. It is of special interest that in *Helminthostachys*, where no secondary thickening is normally found, we can correlate the development of this accessory xylem with the influence exerted by the development of a branch from a vestigial bud upon the completed tissues of the main stele.

Turning now to consider the relations of the branch to the main rhizome, reference may first be made to Text-fig. 5. This represents in outline a transverse section of the main rhizome, showing the branch growing so nearly at right angles to it that its stele is cut longitudinally throughout its course. The xylem is represented in black without any distinction of inner, outer, or accessory xylem. The leaf-trace after the one which subtended the branch is seen nearing the periphery of the

cortex on the right-hand side, and has left an open leaf-gap. The branch stands over the preceding leaf-gap which is now closed. The portion of the branch shown has presumably been laid down by the activity of its proper apex, and has adjusted itself to the position in which the parent fragment lay in the soil. The parent fragment had apparently been inverted in the soil, for while *U* indicates its upper surface, and *L* its lower surface, *u.* and *l.* indicate the upper and lower sides of the branch. The attachment of the stele of the first root of the branch is seen at *r.<sup>1</sup>*, while *l.l.<sup>1</sup>* indicates by a dotted line the course of the first leaf-trace, which was actually shown in a neighbouring section. The branch has acquired a cortex of its own in the outer part of its course through the cortex of the main rhizome, but in the more basal portion the limit is indistinct.

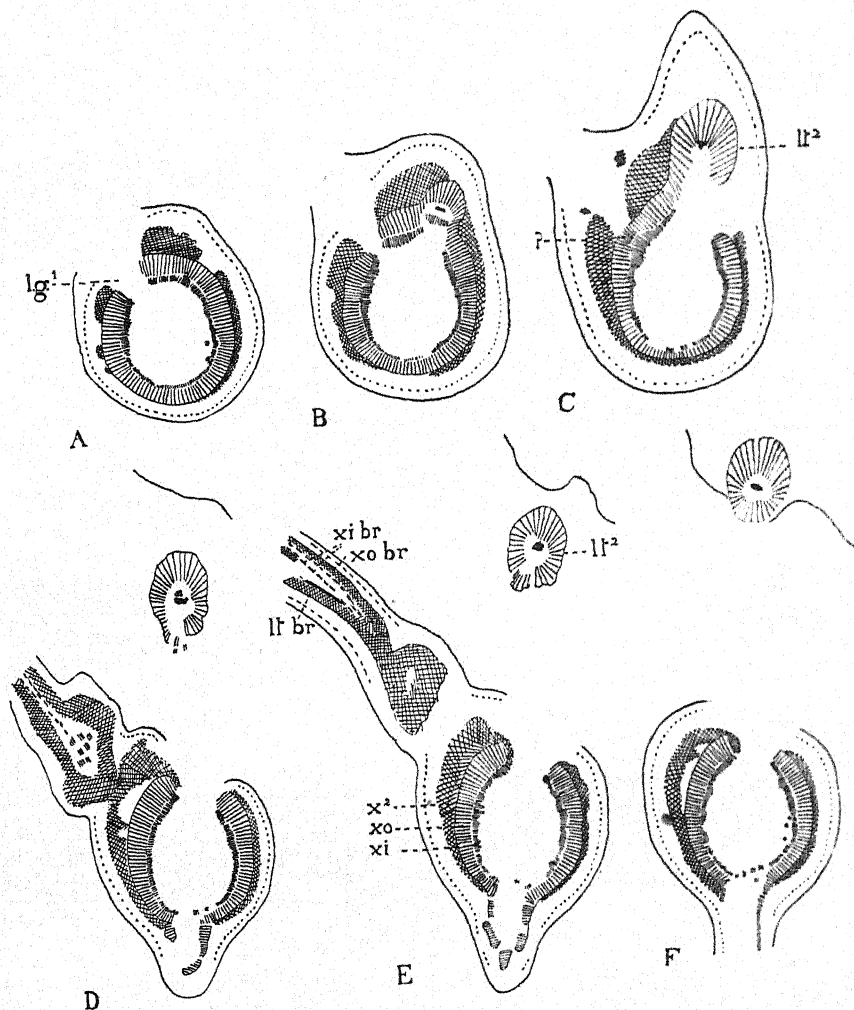


TEXT-FIG. 5. Transverse section of the rhizome bearing the second branch, to show the relative position taken up by the main stem and the branch. Description in text. *U*, upper; *L*, lower side of main rhizome; *u.*, upper; *l.*, lower side of branch rhizome; *r.<sup>1</sup>*, first root of branch; *l.l.<sup>1</sup>*, line of departure of first leaf-trace of branch.

The sections represented in Text-fig. 6, taken together with Photos 27-29 on Pl. II, will serve to illustrate the relation between the branch and the parent rhizome in following the series from behind forwards. Text-fig. 6, A, shows the gap, left by the leaf-trace subtending the branch, beginning to narrow; the leaf-trace has already left the cortex. The xylem tube is seen to consist of inner and outer xylem, and of accessory xylem, the latter being strongly developed at the sides of the leaf-gap. The position of the nascent leaf-trace on the other side of the median line is evident, the trace still forming part of the stellar tube; accessory tracheides are wanting immediately outside the trace. The accessory xylem has been present around the stele since the first indication of the leaf-trace which subtended the gap now beginning to close.

The condition a little further forward is shown in Text-fig. 6, B, in which the nascent leaf-trace is more distinct, the closure of the leaf-gap has advanced, and the accessory xylem is very strongly developed at its margins. The inner xylem is also well developed at the edges of the gap. In the next section (Text-fig. 6, C) the endarch leaf-trace is separating, and on the left-hand side the gap in the xylem has become closed. The inner xylem is of considerable thickness in this region, and has evidently taken a main part in closing the gap. Whether the tracheides in the position marked ? are to be

regarded as inner xylem protruded into the gap, or whether the outer xylem was complete across the gap, could not be determined with certainty. Comparison with the first branching specimen would favour the former



TEXT-FIG. 6. Series of transverse sections of the stele of the second branching specimen, showing the departure of a leaf-trace on the right-hand side, the distribution of the accessory or secondary xylem, and the stages in closure of the leaf-gap on the left-hand side, and the relation to it of the base of the branch stele. The accessory xylem is cross-hatched. Further description in text. *lg¹*, leaf-gap in relation to branch; *lt²*, next leaf-trace from rhizome; *xi*, inner xylem; *xo*, outer xylem; *x²*, accessory or secondary xylem; *?*, possible extension outwards of inner xylem; *xo.br*, outer xylem of branch; *xi.br*, inner xylem of branch; *lt.br*, first leaf-trace of branch.

interpretation. The actual appearance in this critical region is shown in Pl. II, Photo 27, the groups of tracheides which are doubtfully regarded as projecting inner xylem being marked ?. The photograph further shows

the great development of accessory xylem ( $x^2$ ) to the sides of the gap which has just closed, and also the first indication of the development of an arc of xylem connecting the accessory xylem over the region of the gap. In Photo 28, which represents the structure a little further forward, this arc of accessory xylem (marked  $x.o.$  in the upper part of the figure), destined to continue as the outer xylem of the branch, is fully developed, consisting of short reticulate tracheides. Passing into the concavity of the arc of accessory xylem is seen a group of smaller spirally thickened tracheides ( $x.i.$ ) which are continuous above with the inner xylem of the branch stele. The fact that these tracheides of the inner xylem appear to pass out from the region of xylem which was left doubtful at the preceding level (cf. Pl. II, Photos 27 and 28) adds probability to the view that here also the inner xylem was continuous between the main stele and that of the branch, though it is not possible to state this with certainty, as in the case of the first branching specimen. Comparison may be made between the condition shown in Photo 28 for the second branch, with that shown in Photo 23, and in Text-fig. 4, F, G, for the first branch.

Beyond this level the base of the branch stele bends away from the line of the axis of the main stele, as has already been described. The swollen base of the branch stele with its xylem distinguishable into outer xylem (continuous with the arc of accessory xylem) and inner xylem, the origin of which has been referred to above, is still in continuity with the accessory xylem of the stele in Text-fig. 6, D. Still further out, the stele of the branch, which has been seen in Text-fig. 5 to be followed longitudinally, narrows. The connexion between the basal transitional region and the narrow stele is shown in Pl. II, Photo 29, which shows the distinction between the outer xylem of the branch and the tracheides of the inner xylem, forming together with parenchyma a kind of 'mixed pith'; the photograph further shows how the isodiametric tracheides of the accessory xylem are continuous with the elongated tracheides of the branch, where it has grown in length by the activity of its proper apex. The next section of the series (Text-fig. 6, E) is mainly of interest as showing the departure of the first leaf-trace from the branch stele, the direction of which was indicated in Text-fig. 5, *l.l.*<sup>1</sup> The xylem of the base of the branch now appears completely separate from that of the main stele, but the other tissues of the branch still show continuity. In the last section (Text-fig. 6, F) all sign of the branch has gone, the stele of the rhizome is complete, save for the next leaf-gap in relation to the trace which has just left the cortex, but it is noteworthy, as already stated, that the zone of accessory xylem is still well marked, and only ceases to be developed after this leaf-gap has closed.

If the actual connexions of the vascular system of the two branches to their respective steles be compared, an essential agreement will be found

to exist. Omitting some qualifications as to the continuity of the inner xylem in the second specimen made in the detailed accounts above, the important feature appears to be the continuity of the inner and outer xylems from the main stele to the branch. In the case of the outer xylem, this involves the secondary development of accessory outer xylem, which may be limited to the connecting tracts necessary, or may form over the whole stele for a distance of more than two leaf-gaps. Were the branch developed at or close to the apex, the connexions would probably be expressed in a continuity of the primary tissues, as is the case in the vascular disturbance in relation to some vestigial buds. This has been shown above to be comparable with the simpler case presented by the vascular connexions of the first branch. In describing the structure, it is impossible to avoid the metaphor of departure of tissues from the stele, but in interpreting it the other point of view of influences extending backwards from the more or less active bud is essential. Similar considerations had to be entertained in studying the vascular connexions of branches developed from vestigial buds on the rhizome of *Botrychium lunaria*.<sup>1</sup>

### 3. RHIZOMES OF JUVENILE TYPE. PROGRESSION FROM JUVENILE TO ADULT TYPE. CONDENSATION FROM ADULT TO JUVENILE TYPE.

In the preceding pages the structure of rhizomes with medullated and distinctly mesarch steles has been described, the origin of the leaf-trace and its progress through the cortex has been followed, the vascular disturbance of the stele in relation to the vestigial bud has been analysed, and the vascular connexions of branches developed from such buds have been described. This section will be devoted to the structure of the stele and leaf-trace in small rhizomes of the juvenile type, and to the relations between the adult and juvenile types of anatomy.

The available material has consisted of the two small rhizomes arising as lateral branches, of a number of young plants undoubtedly developed from embryos, and of some small rhizomes which might have come either from small branches, or by further development of embryonic plants. The uncertainty (in the absence of the characteristic basal region) is due to the fact that the structure of small branches and of plants of embryonic origin is essentially similar. Branches of *Helminthostachys* have not been described in detail, though Fig. 2 of Farmer and Freeman's paper gives the external appearance of a shoot of this nature, and the small rhizome, the stele of which is figured in Fig. 23 of the same paper, was presumably a branch. The anatomy of young plants has been described by Campbell and by

<sup>1</sup> Ann. of Bot., xxvii, p. 235.

myself. Some redescription is however necessary, since I am unable to agree with Campbell's interpretation of the structure, or with his view of the constitution of the stele of the young and old plants.

*Anatomy of the branches.* Both the branches, the connexions of which with their main stems have been described, were much smaller than their respective parent rhizomes. Their stelar anatomy approximated to that of embryonic plants, but was easier of interpretation, since the vascular elements were more numerous. They had the further interest of a known continuity of the tissues of their steles with the corresponding regions of the parent stele. The first branch was followed in a complete transverse series, while the lower region of the second branch (including the origin of the first leaf-trace and first root) was cut longitudinally, and the remaining portion transversely.

The origin of the first branch has been traced to the stage at which its stele (just before attaining a definite cortex of its own) lay beside the decaying broken end of the parent fragment (Text-fig. 4, J ; Pl. II, Photo 24). The stele of the branch, thus completely organized, had a cylinder of xylem slightly oval in transverse section, and around this a zone of tissue some five or six cells deep, enclosed by the complete endodermis. Presumably pericycle, phloem, and conjunctive parenchyma were represented in the zone between the endodermis and the xylem, and the sieve-tubes soon become recognizable in the transverse section. The chief interest is, however, in the constitution of the xylem. This was solid, in the sense of having no definite pith, though parenchymatous cells were scattered through it. There was a clear distinction between outer and inner or central xylem. The tracheides of the zone of outer xylem (Photo 24, *x.o.*) were larger, and showed a roughly radial arrangement like that found in the outer xylem of the adult type of stele; their walls were pitted. The tracheides of the inner xylem (Photo 24, *x.i.*) were smaller, stained less deeply, and their walls were reticulately or spirally thickened.

Judging by the appearance of the elements of xylem, it would be natural to regard the stele as centrarch, i. e. as having a central group of protoxylem elements surrounded by a zone of metaxylem. The backward connexion of these two components of this stele has, however, been shown to be with the inner and outer (accessory) xylem of the main axis, respectively. That this connexion indicates the right interpretation of the stele of the branch is confirmed as soon as preparations for the departure of the first leaf-trace become evident. A small group of elements now recognizable at the limit between the outer and inner xylem is undoubtedly the protoxylem of the nascent trace, and its position demonstrates the mesarch construction of the small stele. This stage is represented in Pl. III, Photo 30. The leaf-trace xylem is thus derived from a small arc of the outer xylem, with a protoxylem group on its inner face, and as



it separates from the xylem of the stele (Pl. III, Photo 31), the outer xylem becomes at once complete around the inner xylem. A little further on (Pl. III, Photo 32) the leaf-trace and stem-stele are still enclosed by the common endodermis, but the xylem of the leaf-trace has become completed adaxially, in a similar fashion to what has been seen in larger rhizomes. The xylem of the stele is almost solid, with outer xylem surrounding a small central core of inner xylem mixed with parenchyma. This condition is maintained for some distance after the trace has definitely separated from the stele, as is shown in Pl. III, Photo 33, where the section passes through the vestigial axillary bud (*v.*) in relation to the departed trace. The stele already shows the first indication of the position of the protoxylem for the second leaf-trace. The xylem of this trace is seen still forming part of the stele in Pl. III, Photo 34, and it will be evident that a small definite pith is present, the inner xylem being practically replaced by parenchyma. It is unnecessary to follow the anatomy of this branch node by node, especially as it showed no further advance towards the adult type. Indeed, after the departure of the second leaf-trace, the stele, though usually showing a small pith, was smaller than at the base of the branch, and more like that of a young plant.

While the departure of the first leaf-trace from the stele of the first branch has now been examined in transverse section, the corresponding region of the second branch was shown in longitudinal section. The general relations of the inner and outer xylems of this branch to the stele have already been described and discussed. As shown in Pl. II, Photo 29, the base of the branch had a well-developed outer xylem composed of pitted tracheides, and centrally a 'mixed pith', consisting of narrower tracheides, with spiral and reticulate markings, and rather abundant parenchyma. The same structure was traceable as the stele became more slender, and it was evident that this branch, while corresponding in the position of inner and outer xylem to the first branch, tended to have the inner xylem largely replaced by parenchyma. It was possible to prove also, from the longitudinal sections, that the leaf-trace departed from the outer xylem only, and that the stele was to be regarded as mesarch. Pl. III, Photo 35, shows the base of the departing leaf-trace, and it will be clear that while a gap is left for some distance in the cylinder of outer xylem, the inner xylem (*x.i.*), consisting of long spiral tracheides, can be traced straight on within this gap, just as it can on the lower side of the stele. The behaviour of the inner xylem here is thus essentially similar to what held for the first branch, allowing for the fact that in this case more medullary parenchyma is present. The facts seem inconsistent with regarding the centrally placed tracheides as protoxylem, while they agree with regarding it as the representative of the inner xylem, a view which its basal connexion has already suggested.

The stele of the second branch had from its base more central parenchyma than that of the first specimen, and even before the departure of the first leaf-trace may be said to have a definite pith. This is clearly marked above the leaf-trace departure, and it may be noted that nothing in the arrangement of the cells suggests 'intrusive' origin of the pith, which is indeed enclosed within the peripherally placed remains of the inner xylem. A short distance further on (cf. Text-fig. 5) the first root springs from the ventral side of the branch, and from that level onwards the stele is much larger, and has a wide pith surrounded by a tube of xylem. There is little indication of inner xylem in this region, the pith occupying its position, though occasional tracheides occur.

The distal portion of the basal region of this branch, as seen in longitudinal section, naturally corresponds to the appearance of the next portion cut transversely. In Pl. III, Photo 36, the first leaf-trace (*lt*.<sup>1</sup>) is seen just after it has undergone division. The endodermis is complete around the stele, which shows the relatively large pith surrounded by a tube of xylem. This is almost entirely outer (centripetal) xylem, though occasional single tracheides of the inner xylem were found. Dorsally, the xylem for the second leaf-trace is recognizable in the stelar ring, its protoxylem, in the absence of inner xylem, abutting on the pith. The next photographs (Pl. III, Photos 37-39) show the interesting histological changes associated with the departure of this leaf-trace. Photo 37 shows the endarch xylem of the trace just separated from that of the stele. The gap in the tube of outer xylem, which is left by its separation, is partially bridged across by the development of a number of tracheides of inner xylem. In Photo 38 the development of inner xylem across the gap in the outer xylem is much more marked, and inner xylem is also present in the lower part of the stele. The leaf-trace is still within the common endodermis. In the next stage (Photo 39) the trace and stele have their respective endodermal layers complete, the trace shows only slight indications of adaxial extension of its xylem, and the stele has almost returned to the condition from which we started (cf. Photos 39 and 36), in having almost no tracheides of inner xylem developed in the central parenchyma or pith. The position of the protoxylem of the nascent third leaf-trace is recognizable at *px*.<sup>3</sup> The attachment of the second root is seen at the ventral side of the stele in Photo 38.

The rhythm exhibited in the disappearance and reappearance of the inner xylem in this small medullated stele will be discussed later, after the corresponding facts for young plants have been described. The two branches described show agreement in essentials of construction, though medullation by replacement of the inner xylem was earlier and better marked in one case than in the other. Both show, however, that the small stele is to be regarded as mesarch, although the inner xylem consists

of spiral or reticulate tracheides, and may fail to develop in some regions of the stem. The leaf-traces in the first branch showed adaxial completion of their xylem, while in the second branch this was only slightly indicated. Another interesting difference, for comparison with young plants, was that the first leaf on the one branch was an arrested structure, while in the second branch it was shown by its vascular supply to have been normal.

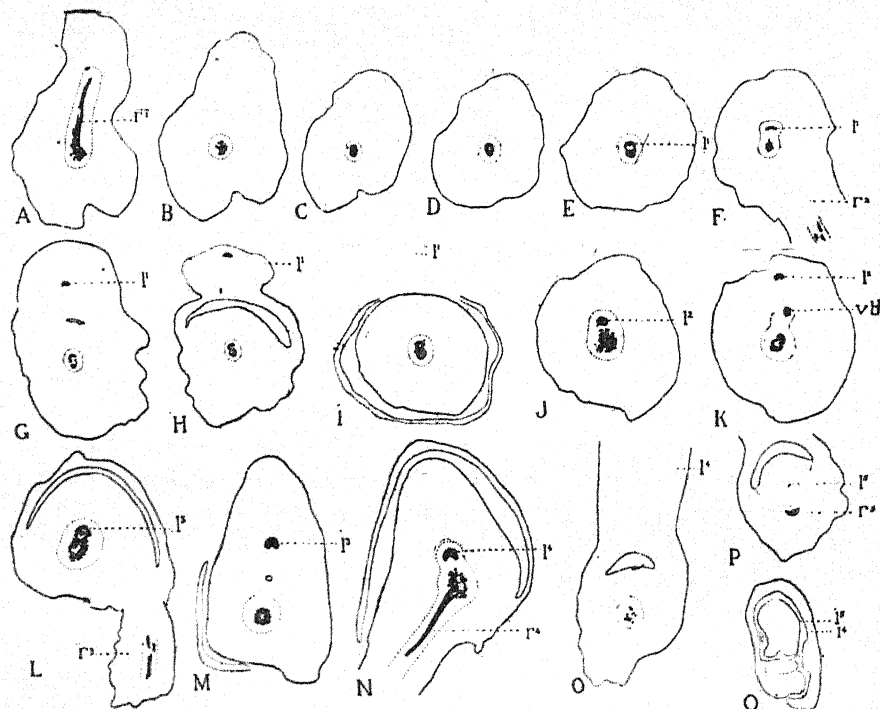
*Anatomy of young plants.* The anatomy of young plants developed from embryos will be dealt with as briefly as possible. The main facts are recorded by Campbell and myself in the existing literature, but not in the form required for comparison with the branches and the rhizomes of adult type. What is further necessary may be brought out by following a series through part of a well-developed young plant, such as that figured in outline in the previous paper of this series.<sup>1</sup> Such a plant would show the large foot at the base, the primary root with its vascular system continuous with the base of the stem stele, but not in the same straight line as this, and the strictly dorsiventral construction of the further growth. The plant actually selected for description had borne four expanded leaves dorsally, and a number of roots (in addition to the differently placed primary root) ventrally.

Transverse sections of this plant at various levels are represented in Text-fig. 7, the external outline, the endodermis, and the xylem only being shown. The scale did not allow of a distinction being made in these figures between the inner and outer xylem. In Text-fig. 7, A, the continuity between the stele of the first root and the base of the stem-stele is seen, and this root is seen to differ from all the later ones in standing on the morphologically dorsal side of the plant. B and C show the hypocotyledonary stele, while D, E, F, G show the departure of the first leaf-trace, the slit leading down to the first vestigial bud being evident in G. The second root is seen in F. The departure of the second leaf-trace extends from H to K, the last section passing through the vestigial bud; L shows the third root and the preparations for departure of the third leaf-trace, while in M this trace is seen with its axillary canal. In N the fourth leaf-trace is distinct from the stele, and the attachment of the fourth root is seen. Beyond this, the stele passes into a more meristematic condition, the last tracheides visible in P being a dorsal group in relation to the young fifth leaf, and a ventral group belonging to this region of the stele; in connexion with this, the fifth root is developing.

The more important structural features of the stele of this plant are illustrated by Photos 40-46, on Pl. III. The xylem of the hypocotyl exhibited a central group of tracheides surrounded by a more or less definite zone of somewhat larger tracheides. This zone is rather irregular at the level figured (Pl. III, Photo 40), but became more regular and closer

<sup>1</sup> Ann. of Bot., xxviii, p. 30, Text-fig. 9 A.

a little higher up, before any indication of the departure of the first leaf-trace was evident. Longitudinal sections of this region in other plants showed that the central xylem consisted of spirally thickened tracheides, while the peripheral ones were pitted. Owing to this structure, the stele was described in my earlier accounts as centrarch, the inner xylem being regarded as protoxylem, which it resembles histologically. For reasons that will be further evident below, I am now led to distinguish even in these slender steles between inner and outer xylem, as in the case of the branches previously described, and to correct in this sense my earlier statements.



TEXT-FIG. 7. Series of transverse sections of the rhizome of a small plant, developed from an embryo, to show the relative positions of the various organs. The outline of the rhizome is given, that of the stele is a dotted line, the xylem is black. Description in text.  $l^1, l^2, l^3, l^4, l^5$ , successive leaves or leaf-traces;  $r^1, r^2, r^3, r^4, r^5$ , successive roots;  $vb^2$ , second vestigial bud. The slits leading to other vestigial buds are also shown.

The stelar xylem in the hypocotyl can be regarded, as in the further regions of the shoot, as composed of a dorsal portion destined to continue outwards as the first leaf-trace, and a ventral portion which is more strictly cauline. At the level of the section represented in Pl. 3, Photo 41, the arc of outer xylem destined for the leaf-trace is rendered distinct from the lower portion of the stele by the group of parenchyma immediately within its endarch protoxylem. The true protoxylem for the trace was evidently placed immediately within the outer xylem, thus indicating the mesarch

nature of the stele in the hypocotyl. On the separation of the leaf-trace xylem (Pl. III, Photo 42), the outer xylem becomes complete around the inner xylem of the stele, which again forms a solid cylinder. For some distance the stele, now belonging to what may be termed the first internode of the epicotyl, maintains this appearance. Then parenchyma again appears in the central xylem, replacing many of its elements, and shortly after this the second leaf-trace becomes clearly recognizable, forming the dorsal half of the stele (Pl. III, Photo 43). In this section the xylem of the leaf-trace is again seen to be endarch, and to be continuous with the outer xylem of the stele. This outer xylem is one or two tracheides thick in the lower portion of the stele, and surrounds the small 'mixed pith', consisting of a number of parenchymatous cells, and some three or four tracheides of the inner xylem (*x.i.*). The separation of the leaf-trace xylem would evidently leave a gap in the outer xylem, but before this takes place (Pl. III, Photo 44) the inner xylem is found to be much more strongly developed, rendering the stem portion of the stele almost solid again. Indeed, before the leaf-trace xylem completely separates (Pl. III, Photo 45), the outer xylem in the stem stele becomes continuous, so that on the departure of the trace, the stele is left with a complete solid xylem strand consisting of inner and outer xylem. In the preparations for the departure of the third leaf-trace medullation again takes place by replacement of the inner xylem by parenchyma. Thus in Pl. III, Photo 46, the xylem of the nascent third trace is recognizable, forming the dorsal portion of a stele that has a small pith, scattered tracheides of inner xylem round this, and a continuous tube of outer xylem.

It is unnecessary to follow the changes node by node, for the facts just given will be sufficient to show the rhythmical nature of the changes in the stele associated with the leaf-trace departures. It should be mentioned, however, that this stele never became quite solid above the departure of the third trace, a small pith persisting. The second root arose from the ventral surface of the rhizome, just after the first leaf-trace separated from the stele, and the third root was inserted shortly after the stage reached in Pl. III, Photo 46. The leaf-traces of this plant were endarch, and showed no adaxial completion of the xylem, but cases in which this was effected have been observed in other young plants.

*The progression from the juvenile to the adult type of rhizome.* The comparison of the stellar structure of young plants and branches on the one hand, and of rhizomes of different diameters, but with the usual adult type of stele, on the other, reveals an essential correspondence. The chief differences in the adult type, apart from size, concern the definite and persistent medullation; the development of the elements of the inner xylem as pitted tracheides and the occurrence of an internal endodermis.

The origin of the pith in the seedlings and other small rhizomes

studied shows no indication of being intrusive. The pith arises by the greater or less development of parenchyma in the central region of the stele. There is no indication of conversion of tracheidal elements to parenchyma, but this phrase expresses in a general way the mode of origin of the pith, since different plants or regions exhibit completely solid inner xylem, inner xylem with scattered parenchymatous cells, mixed pith with parenchyma predominating in the central region, and lastly a small pith where the central region is free from tracheides. It may therefore be stated definitely that a pith, such as that shown in Pl. III, Photos 36, 39, 46, is purely intrastelar and in no way due to intrusion.

None of the seedlings, branches, or juvenile rhizomes of uncertain origin showed the actual progression to the fully adult type of stele with an internal endodermis, but comparison of rhizomes of different sizes of both juvenile and adult type gave a series which lent no support to the view that at a later stage of development an intrusive pith was present in addition to the primary intrastelar pith. To draw the line on the appearance of an internal endodermis would be wholly artificial, as this may be present, incomplete, or absent in rhizomes of various sizes. The value of the internal endodermis in this plant as an indication of the limit between stele and intrusive cortex is further discredited by the occasional appearance of an internal endodermis in the base of roots, as described earlier in this paper.

With regard to the change in inner xylem from spiral or reticulate to pitted tracheides, the latter characterizing the inner xylem of the adult type of rhizome, the evidence of direct transition in any one piece of rhizome is also lacking. But it has been shown, in considering the branches and young plants, that the inner xylem of the juvenile rhizome behaves like the inner metaxylem of the adult rhizome in the region of the leaf-gap, and also that in the case of branches there was continuity between the inner xylem of the main rhizome and the branch. The question of the transition between the two histological types in the ontogeny, though it would be interesting, is not vital to the morphological interpretation of the stele. That there is a transition at a certain stage may reasonably be assumed.

Since none of the slender rhizomes of juvenile type, though some of them consisted of a considerable number of nodes, showed the advance to the adult type, it is clear that this is not necessarily (or perhaps even usually) attained quickly. In one piece of rhizome, however, a rapid transition from the condition with a stele with a solid xylem to the fully adult structure was exhibited, and, though peculiar in some ways, this rhizome may be briefly described. It was probably a branch, since, while much thicker than any young plant, the first leaf-trace was imperfectly lignified and supplied an arrested leaf. The character of the leaf-traces and their departure have already been described (see Pl. I, Photos 14-19), and

all that need be done is to refer to the progress in size and in stelar complexity.

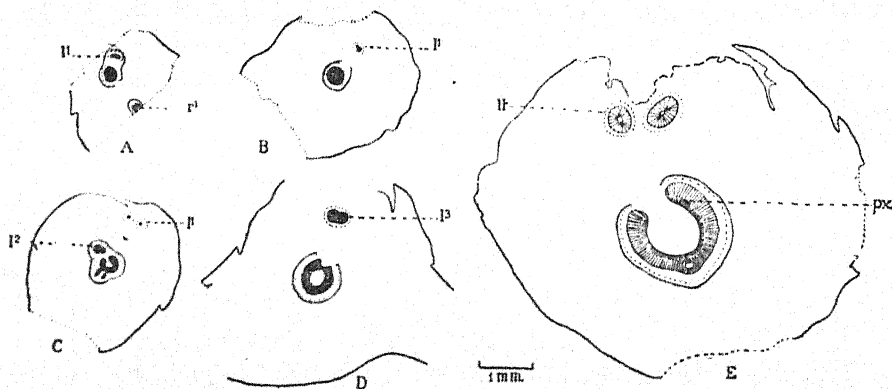
This piece of rhizome measured about an inch and a half in length, and rapidly increased in diameter from the narrow basal end till its full diameter was attained and then maintained. Its diameter at the base was slightly over 2 mm. and the diameter of the adult region was about 7 mm. A transverse section at the base (Text-fig. 8, A) showed the xylem of the first leaf-trace already separate from the stelar xylem, but within the common endodermis. The xylem of the stem stele was complete, showing no indication of a gap, and at this level was practically solid, only a few parenchyma cells being scattered among the more central tracheides. There were indications of a distinction between somewhat smaller tracheides of the inner xylem and the zone of outer xylem, though the limit was not easy to decide definitely from transverse sections. Where the sections were slightly oblique, it could be seen that the inner tracheides were spirally thickened and those of the outer xylem pitted. The stele at this level, though larger than the other examples described, was thus of the juvenile type. The xylem of the departing first leaf-trace was clearly endarch. It did not show adaxial completion, and indeed as it became further removed from the stele ceased to be lignified; it was evidently related to an arrested leaf.

Meanwhile, parenchyma had become more definitely present in the central region of the stele, replacing some of the inner xylem and forming a small pith, around which a broken ring of elements of the inner xylem remained. The distinction between the outer and inner xylem was evident on the appearance of the protoxylem of the second leaf-trace, which had elements of inner xylem internal to it. Then the inner xylem disappeared opposite to the nascent endarch trace, the pith being continuous into the concavity of the latter. The xylem of the trace, derived from the outer xylem only, almost closes round as it separates from the xylem of the stele (Text-fig. 8, C). The adaxial closure becomes complete later, and this trace passed through the usual clepsydroid stage and divided just before it departed from the cortex. While the separation of the second trace has been proceeding, the stele has enlarged and is provided with a greater definite pith. The inner xylem is especially well represented as the gap closes.

It is unnecessary to follow the elaboration of the stelar structure further in this rhizome, but this portion is of interest in showing how, between the departure of the first and second leaf-traces, the xylem passed from the solid condition with inner and outer xylem to the definitely medullated condition found in small rhizomes of the adult type of structure. It is impossible to state at what level the inner xylem was composed of pitted tracheides, but it was evidently by now or not much further on. The

relative size of the rhizome and stele after the departure of the third leaf-trace is seen in Text-fig. 8, D. The rapidity of the transition in this rhizome can be associated with the greater size of the stele even at the base, and presumably with more effective conditions of nutrition. The comparative sizes of stele and rhizome from the base to the attainment of the full diameter can be followed in the diagrams in Text-fig. 8. When the full size was attained (Text-fig. 8, E) the stele showed the size and complexity of the fully adult type, but had no internal endodermis.

*Condensation of stelar structure from the adult to the juvenile type.* It is usual to find the primary structure of a young plant or a branch continuing on the up-grade of elaboration until the adult type is reached and then maintained. It was of interest, as bearing on the significance to be



TEXT-FIG. 8. Selected transverse sections to show the progression in size and stelar complexity in a rapidly widening piece of rhizome. A, B, showing departure of the first leaf-trace; C, at the level of the first vestigial bud and showing the second leaf-trace on the left; D, the leaf-trace at the clepsydroid stage; E, the full-sized rhizome showing a dividing leaf-trace at the periphery of the cortex.  $l^1$ ,  $l^2$ ,  $l^3$ , leaf-traces of the first three leaves;  $r^1$ , stele of the first root;  $l$ , dividing leaf-trace of full-sized region;  $px$ , protoxylem group of next leaf-trace.

attached to this progressive elaboration of the stele in the ontogeny, to find a converse change exhibited by certain rhizomes, in which a condensation or reduction from the adult to the juvenile type of stele could be traced. This was associated with diminution in size of the rhizome as a whole, and may reasonably be regarded as due to growth under less favourable conditions of nutrition.

The amount and nature of the condensation will be evident on comparing transverse sections of the stele of the same rhizome at different levels, photographed to the same scale. Thus Photos 51 and 52 on Pl. III are respectively further from and nearer to the apex of the same piece of rhizome. The former shows the usual structure of a small rhizome of adult type, with a definite pith surrounded by a xylem-tube with well-developed outer and irregular inner xylem. This rhizome actually had an imperfect internal endodermis. Passing towards the apex of the piece of rhizome



there was a progressive diminution in thickness, and the stele exhibited changes similar to those seen in the ascending series of complexity traced in rhizomes of increasing size, but in a converse direction. At the level shown in Photo 52 the stele agreed in size and organization with those of small branches or young plants in which the scattered elements of inner xylem formed, with the cells of the central parenchyma, a mixed pith. The stele had returned to the juvenile type, and the changes in relation to the departure of a leaf-trace agreed with this. A corresponding, though less extreme reduction, is seen on comparing Photos 48 and 50 from another piece of rhizome. Both pairs of photographs show that there is a corresponding reduction in the size of the leaf-trace.

Such specimens seem to indicate clearly that the simpler type of stele characteristic of normal young stages in the ontogeny of *Helminthostachys* is to be associated with small size and less efficient nutrition. The same juvenile structure can be resumed when from any cause an adult rhizome becomes again feeble and more slender.

#### 4. ON THE INTERPRETATION OF THE CONSTRUCTION OF THE RHIZOME AND STELE IN *HELMINTHOSTACHYS*.

In the preceding parts of this paper a number of facts have been described which amplify our knowledge of the anatomy of *Helminthostachys*. In the description of the facts theoretical interpretations have been touched upon as lightly as possible. Some general considerations may fitly be placed here, and are necessary in order to properly discuss Professor Campbell's views on this plant.

If the descriptions of the adult rhizome, the branches, and the young plants are compared, an essential similarity in construction will be found to underlie the differences presented by rhizomes of various sizes. As regards general morphology the rhizome is throughout dorsiventral, the ventral region bearing roots but no leaves, while the dorso-lateral leaves alternate, as shown by their leaf-traces, to either side of the middle line. In relation to each leaf is its vestigial axillary bud, which may be more or less displaced in front of the leaf-trace—in adult rhizomes usually by the whole length of the leaf-gap. There is thus a general indication of a segmental repetition of the parts of the shoot which will need to be considered further below.

The essential agreement in stelar construction between the juvenile and adult rhizomes must first be made clear. If the series through the adult rhizome (Text-fig. 2) be considered, it will be evident that while the large pith is continuous from node to node it is unequally encroached upon by xylem at different levels. This especially concerns the inner xylem. The inner xylem, on the one hand, diminishes in amount (and often disappears),

internal to the protoxylem of a nascent leaf-trace, by the development of parenchyma replacing more or less completely the xylem elements. On the other hand, the pith is diminished immediately above a leaf-gap by an increased development of inner xylem forming a marked bulge inwards.

When this rhythm is considered in the light of the stelar structure of small rhizomes it gains in significance. Thus in steles with a small pith like that shown in Photos 36-39 we find the inner xylem least developed just before the leaf-trace begins to separate, while on the separation an increase of the inner xylem takes place, which is especially marked on the dorsal side of the stele below the gap in the outer xylem. In this case, as in the adult rhizome, the pith persists even where the inner xylem is at its greatest development, and this is on the dorsal side of the stele below the closing leaf-gap. In other cases, and especially in the basal nodes of the young plant (Photos 42-46), the inner xylem is less developed as the trace prepares to separate, and the subsequent increase of inner xylem leads to total filling up the centre of the stele so that the pith is interrupted at the base of each internode.<sup>1</sup> Whatever be the ultimate explanation of it, the rhythm in proportional development of inner xylem and pith is the same in large and small rhizomes.

The pith can be seen to arise in *Helminthostachys* by the increase in the amount of parenchyma in the centre of the stele. Usually scattered elements of parenchyma are present in the inner xylem, though this may consist of tracheides only, and all grades between this and the complete replacement of inner xylem by parenchyma may be found in juvenile steles. The rhythmical variation in amount of the inner xylem appears in some cases to negative the idea of the pith being of 'intrusive' origin. Thus, in such cases as those shown in Photos 35 and 38, the pith is cut off from the outside by the inner xylem,<sup>2</sup> the leaf-gap only affecting the outer xylem. It is only in the adult rhizomes with larger leaf-gaps that continuity between the cortical and medullary parenchyma is marked, and it is in these regions that an internal endodermis is often developed. The appearance of this is not suggestive, however, of its being a morphological limit between an intrusive cortical pith and the stele;<sup>3</sup> the occasional presence of an internal endodermis in the root is further evidence against attaching morphological importance to it in the stem. A still further argument against regarding the pith as extra-stelar is afforded by its reduction and the distribution of tracheides through it when the stele exhibits condensation from the adult to the juvenile type (cf. Photos 51, 52).

Though the inner xylem differs in its histological features in the

<sup>1</sup> A similar return to a solid stele after a leaf-trace departure is described for a small plant of *Botrychium lunaria* by Bower (Ann. of Bot., xxv, p. 541).

<sup>2</sup> Cf. *Botrychium lunaria*, Ann. of Bot., xxvii, p. 221, Pl. XX, Photo 14, and the discussion and comparison with *Osmunda* there.

<sup>3</sup> A similar conclusion was reached in the case of *B. lunaria*. Ibid., p. 217.

juvenile and adult rhizomes, critical consideration shows that even the small rhizomes with their central elements spirally thickened are to be regarded as mesarch. The mesarchy is shown where the protoxylem for the leaf-trace appears (cf. Photo 30). This interpretation is supported by the behaviour of the spirally thickened tracheides of the inner xylem at such leaf-trace departures as those in Photos 35, 37, and 38, a behaviour quite inconsistent with regarding them as protoxylem.

An essential similarity is thus seen in the small and large steles, though the actual construction differs in rhizomes of different size. It is usual to find the primary structure of a young plant or a branch continuing on the up-grade of elaboration until the adult type is reached and maintained. This is natural and intelligible whether the ontogenetic progression is to be looked upon as a phylogenetic recapitulation or as an expression of the cumulative increase in strength and nutritive power of the plant. The fact that in *Helminthostachys* the earlier grades in the progression may be maintained for many nodes of a rhizome, or, on the other hand, may be rapidly passed through, suggests that the physiological explanation may be the more important. This is further shown by the juvenile structure being resumed when rhizomes of adult type become more slender under unfavourable conditions of nutrition. Thus the usual progression must be regarded as an expression of physiological differences, and its use as an index of phylogenetic history must be critically considered in this light. In *Helminthostachys* at least the physiological interpretation seems inevitable, whatever phylogenetic value may be attached to the different manifestations of the underlying type of construction.

The essential structural fact appears to be the existence of two components in the xylem, inner xylem and outer xylem. Various indications suggest that this may be a distinction of fundamental importance in the general morphology of the conducting system of a shoot. Not to go into detail here, it may be mentioned that a similar distinction has been made in the case of the independently evolved conducting system of Mosses,<sup>1</sup> and that it is exhibited by the stele of the Lycopodiales, Equisetales, and Sphenophyllales. It is present in the stele of the adult stem in some Ferns (e.g. Gleicheniaceae) and is indicated in others. The distinction is clearly suggested in the slender basal region of the stems of many young Ferns. But the most interesting cases in the Pteropsida are found in a number of relatively archaic groups, such as the Osmundaceae, the Coenopterideae, especially the Zygopterideae, and also in some Cycadofilices.

Leaving phylogenetic considerations out of the question for the present, the Ophioglossaceae appear to find their place with the last-named groups, as regards the general comparative morphology of the stele. The Ophioglossaceae are of peculiar interest on account of the range of variants of the

<sup>1</sup> Cf. Tansley and Chick: *Ann. of Bot.*, vol. xv, p. 1.

same essential type of primary construction they present. In *Helminthostachys* the inner xylem is better represented than in any other existing medullated plant. Indications of inner xylem are found in *Botrychium*, and a mixed pith may occur as a result of traumatic disturbance. In *Ophioglossum* tracheides may be developed throughout the pith in branching rhizomes, while strands of tracheides occur normally in the pith of some specimens of *Ophioglossum pendulum*.<sup>1</sup>

In attempting to get a deeper insight into this distinction of inner and outer xylem, it must be borne in mind that the descriptive statement that a leaf-trace 'departs' from a stele is metaphorical. The vascular system has no such individuality or power of branching. In the light of development it must be regarded as laid down along certain tracts, determined partly by the meristematic construction, and partly by influences proceeding backwards from the growing points of the main axis, or of lateral leaves or branches. While our knowledge of the necessary facts is too imperfect to make any inference more than tentative, it is suggested that the central region of a stele may be directed from the main apex, while the peripheral region is largely influenced from the developing leaves. In support of such a conception of the vascular structure, it may be pointed out that the study of the Ophioglossaceae has shown clearly the reality of such backward influences along more or less predetermined tracts, in the case of the vascular connexions of the branches. This was evident in the study of the branches of *Botrychium lunaria*, and has been shown above for *Helminthostachys*. In the latter plant we see further that such an influence may extend to the main stele, and modify its structure all round and for a considerable distance; the extensive development of accessory or secondary xylem in the second branching specimen was clearly due to the influence of a developing branch upon the mature primary structure of the stele.

In the preceding considerations, the stem with its stele has been regarded as the unit. It is possible, however, to regard the rhizome of *Helminthostachys* as exhibiting a segmental construction from three rows of segments, two dorso-lateral and one ventral. The distinction of the dorso-lateral segments is pretty clear, since each bears a leaf and its related vestigial bud; the distinction of the ventral segments which do not bear leaves, and on which the roots are borne, is less evident. Such a construction differs in the existence of a ventral region not having leaves, from the radial construction exhibited by *Botrychium*, where each segment bears a leaf and a bud. The difference here shown between these dorsiventral and radial types of rhizome is of course present in Ferns at large, but this preliminary consideration may be confined to the Ophioglossaceae.

The radial and strictly dorsiventral types of shoot, with the general appearance of a segmental construction, as seen in *Botrychium* and

<sup>1</sup> Petry: Bot. Gazette, vol. lvii, p. 183, Figs. 12, 13.

*Helminthostachys*, are paralleled by the independently evolved shoots of the sexual generation of Mosses and leafy Liverworts respectively. In the case of the Bryophyta we know that the construction of the mature shoot is directly related to the apical segmentation. To what extent such a relation holds in the case of the more massive shoots of Ferns is, in my opinion, an open question which requires fuller investigation. The view has been expressed on the one hand that the leaf-development in Ferns is independent of the apical segmentation.<sup>1</sup> On the other hand, we know that in certain cases, such as *Ceratopteris*, the relation between apical segment and leaf holds, and this is also stated to be the case for the dorsiventral rhizome of *Polypodium vulgare*.<sup>2</sup> The appearance of the mature regions of both radial and dorsiventral shoots is at least consistent with assuming such a relation to hold, and is otherwise insufficiently explained. If such a segmental relation holds for *Helminthostachys*, for example, it would explain the regular succession of the lateral members in a way that does not involve the acceptance of any view that denies the existence of an axis. On such lines we should be prepared to find that the stele of the rhizome could be regarded, in part at least, as cauline, though influenced profoundly by the leaves.

The segmental construction with two rows of dorso-lateral and one row of ventral segments is indicated, not only in the general morphology of the rhizome of *Helminthostachys*, but also in the stelar structure. It is shown most clearly in smaller rhizomes, where the whole of each dorso-lateral segment of the outer xylem of the stele is continuous with the outgoing leaf-trace. Since the segmental construction is of the rhizome as a whole, the vascular system being developed along certain tracts through this, only two of the segments may be evident in the stele itself, the third being indicated by the departing leaf-trace. In the less extended adult type of stele all three segments are represented in any one section.

Thus the three components of the stele are represented in a large rhizome, such as that in Text-fig. 1, B, by the ventral portion where the roots are borne, the right dorso-lateral portion concerned with the vestigial bud, and the left dorso-lateral portion concerned with the next leaf. In small steles the distinction of three parts is shown in Photo 32, where the ventral portion and the right dorso-lateral portion are evident in the stele of the stem, while the left dorso-lateral segment is represented by the departing leaf-trace. The same thing is shown in Photo 52, where the dorso-lateral segments are indicated by the leaf-trace (*Lt.*), and by the region with the protoxylem group (*px.*) of the next trace already evident. The rest of the stele corresponds to the ventral segment.

If the stele be traced into the meristematic region behind the apex,

<sup>1</sup> Cf. the discussion of this question in the Land Flora, pp. 175-7.

<sup>2</sup> Klein: Bot. Zeit., 1884, p. 646.

the same distinctions can be drawn so far up as the procambial strand exhibits any lignified elements. Pl. III, Photo 53 shows a transverse section of a stele at this level; the outline of the procambial cylinder can be traced, and in it two groups of protoxylem elements are lignified; one of these marked *lt.* corresponds to the leaf-trace supplying the youngest developing leaf, while the other (*px.v.*) is the early formed xylem of the ventral segment. A corresponding independence of the ventral region of the xylem has been traced to close behind the apex in a number of small rhizomes.

We are now in a position to consider the divergent views expressed by previous investigators of *Helminthostachys*, as to the evidence for or against the stele, or a portion of it being cauline.

According to the work of Farmer and Freeman<sup>1</sup> on adult rhizomes, the stele can be followed to the apex of the stem beyond the youngest leaves and roots, and hence is cauline. These investigators further emphasize the evidence afforded by the ventral side of the dorsiventral rhizome, from which leaves are absent, while the vascular tissue can be traced up to the apical meristem. With this view, according to which there is a stem with a stele which in part at least is strictly cauline, I am in essential agreement.

Campbell, who has investigated the question mainly on young plants, a number of which he describes in some detail, comes to a very different conclusion, which will be clear from two quotations, remembering that he takes as his starting-point 'the single axial strand of collateral structure throughout cotyledon and root', as found in the young plants of some species of *Ophioglossum*. From such strands he derives directly the dictyo-stelic arrangements in *Ophioglossum* and Marattiaceae. After considering these he continues: <sup>2</sup> 'The second type of skeleton is that found in *Botrychium* and *Helminthostachys*. This is a solid, hollow cylinder, with inconspicuous leaf-gaps, resulting from the union of the broad leaf-traces which fuse completely to form this hollow stele. That the cylindrical bundle, or siphonostele, is not due to the formation of a pith within the protostele is clearly shown by the development of the bundle in the young sporophyte of *Botrychium*, where it can easily be seen that the component bundles are separate at first, and that the pith, so called, of the siphonostele is merely a portion of the ground tissue that is included between them, and which later becomes entirely separated from the cortical tissue. A similar condition of things may be found in tracing the development of the vascular cylinder in the young stem of *Helminthostachys*.'

In describing the structure near the tip of the young rhizome of *Helminthostachys*, Campbell says<sup>3</sup> earlier in the same work: 'From this study of the development of the leaf-traces, following them from the stem

<sup>1</sup> Loc. cit., p. 428.

<sup>2</sup> Loc. cit., p. 214.

<sup>3</sup> Loc. cit., p. 78.

apex downwards, it appears that the cylindrical stele in *Helminthostachys* arises in precisely the same way as that of *Botrychium*, viz. by the union of the leaf-traces. The appearance of procambium upon the ventral side of the stele, which in longitudinal section appears to be derived directly from the stem apex, can thus be explained by the ventral extension of the broad leaf-traces, which meet on the lower side of the stem as well as above, and the cylindrical stele is thus developed. It is not impossible that the root-traces may also contribute to some extent to the development of this ventral portion of the stele.<sup>1</sup>

If the last sentence is taken to mean that a ventral portion of the stele not derived from leaf-traces is always present, this view would not differ so fundamentally from the segmental analysis of the rhizome and stele indicated above, though the idea of root-traces 'contributing' to the stele is even less satisfactory than that of entering leaf-traces.<sup>1</sup> It is clear, however, from the context, from the description of particular plants, and from the view of the nature of the pith expressed in the first quotation above, that Campbell recognizes steles of young plants as resulting entirely from the union of successively older leaf-traces. This view leads him to interpret certain structural appearances in a way that I am unable to accept.

Thus, if the description of a young plant given on pp. 71-3 of 'The Eusporangiatæ' and illustrated by Figs. 49 and 50 be referred to, it will be found that the anatomical relations of the leaves and stems are followed from above downwards. The bundle from the youngest leaf is traced inwards from above the level of the stem apex to a plane below this. Not even a meristematic or procambial equivalent of the stem stele is recognized as present below the apex for the trace to join on to, but this leaf-trace is regarded as forming the whole of the stele present in the rhizome below the apex. On to this the trace of the next older leaf is followed, so that the stele below is regarded as made up by the union of the first and second leaf-traces, no stem portion other than this being recognized. The double nature of the xylem in this stele-below the entry of the second trace is figured in detail (Fig. 50), and explained as being due to the portions of xylem derived respectively from the two leaf-traces.

The difficulties which have led to this interpretation of the facts seem to be due partly to the extreme closeness of the apex to the youngest developing leaf, and partly to the fact that the actual apex is sunken and ventrally displaced. Thus the leaf-trace is met with above the level of the apex, and below this level may be lignified, while the cauline component of the stele is still procambial. The double nature of the xylem of the stele behind the junction of a leaf-trace is readily seen, and is accurately figured by Campbell, but the lower of the two components is not traceable to the next younger leaf, but, as shown above, is the ventral and cauline

<sup>1</sup> Roots and leaves might both be regarded as influencing the differentiation of the stele.



portion of the stele. This is most clearly seen on following the changes in the stele from below upwards, and this method also shows the intrastelar development of the pith which is naturally called in question on Campbell's view.

A further argument against Campbell's interpretation is afforded by the vascular structure of the hypocotyl. In this region also the stele appears composed of a dorsal portion continuous with the first leaf-trace, and of a ventral portion. Apart from the fact that the ventral portion is not traceable into the second leaf (as would be necessary on Campbell's view), it would appear a strained interpretation of the ventral and often larger portion of the stele below the first leaf, to regard it as due to the vascular strand entering from the next younger leaf; the latter was probably not developed when the vascular structure of the hypocotyl was determined.

This question has been discussed at some length because the interpretation of the vascular structure of the *Ophioglossaceae* given by Campbell has wide bearings on the general morphology of vascular plants. His view is in accordance with a phytonic theory, and while applicable with some difficulty to the stelar structure in *Ophioglossum*, meets with greater difficulties in the stele of the shoot of *Botrychium*, which is also radially constructed. It appears to be almost negatived when the attempt is made to apply it to the dorsiventral shoot of *Helminthostachys*, where there is a ventral component not bearing leaves.

On the alternative view held in this paper, the segmental construction of the shoot is recognized, but is not regarded as inconsistent with the primary existence of an axis upon which the leaves arise as lateral appendages. Both the segmental construction of the shoot and its morphological unity require to be recognized. Phytonic theories may be said to over-emphasize the first feature, while opposing views tend to minimize the reality of segmental construction. This general aspect of the question cannot, however, be further discussed here.

##### 5. CONCLUDING REMARKS.

In the first number of these studies,<sup>1</sup> the direction which a number of features of the rhizome of *Botrychium lunaria* indicated for the relationship of the *Ophioglossaceae* to other plants was very briefly considered. The general view expressed was that 'the stelar structure, the medullation, the construction of the leaf-trace, and the nature of the branching, are all consistent with a relationship of the *Ophioglossaceae* to the ancient Fern stock, the general features of which are indicated in the relatively primitive groups of Ferns, such as *Zygopterideae*, *Botryopterideae*, *Osmundaceae*, *Hymenophyllaceae*, &c.' This is in general accord with the views expressed by Renault and Scott,<sup>2</sup> and with the position now given to the group by

<sup>1</sup> Loc. cit., p. 240.

<sup>2</sup> Studies in Fossil Botany, p. 640.



Bower,<sup>1</sup> and will not be further discussed here. It is confirmed by this study of *Helminthostachys*.

Without dwelling on the question of actual relationship of the group, some of the main data for comparison afforded by this plant may be reviewed, and possible comparisons indicated. In doing this it is necessary to extend the field of comparison to some of the Cycadofilices. The main features of importance for comparison in the rhizome of *Helminthostachys* are (a) the presence of inner and outer xylem, (b) indications of secondary thickening, (c) peculiarities of the leaf-trace, (d) axillary branching and the vascular relations of the branch. These may be briefly discussed in order. The comparisons indicated are not to be taken as necessarily implying actual relationships.

(a) The distinction of inner and outer primary xylem is, as has long been known, exceptionally clear in the large adult rhizomes of *Helminthostachys*. An essentially similar construction has been shown in this paper to hold for small rhizomes, where, however, the inner xylem does not consist of pitted tracheides; it may be solid or form a mixed pith. The progressive elaboration exhibited by the stele of *Helminthostachys* is particularly instructive, as the zone of inner xylem is preserved even in the large rhizomes. Thus comparing *Helminthostachys* with other Ophioglossaceae we have an interesting range of variants on a fundamental type of construction. In the one direction we have the *Ophioglossum* type with a reticulum of collateral bundles, consisting of outer primary xylem and phloem, all trace of inner xylem having usually disappeared. On the other hand, we have the *Botrychium lunaria* type with only traces of inner xylem round the pith, but with a well-marked zone of primary outer xylem passing on the outside into the zone of secondary xylem in large stems. In other species of *Botrychium*, e.g. *B. virginianum*, the radial arrangement of the elements of xylem begins at once, so that in this respect no equivalent of the primary outer xylem of *B. lunaria* or *Helminthostachys* is recognizable. In the species of *Botrychium* we find the same problem of what is to be regarded as primary xylem that faces us in the higher Gymnosperms. The same general direction of elaboration of a monostele appears to have been followed independently in several lines of Filicales, and in the Lyginodendreae, while parallels can be traced in other groups such as the Lepidodendreae.

As to how far the distinction of inner and outer xylem can be usefully followed in the Filicales, cannot be discussed here. Among existing Ferns it is clearly seen in such forms as the Gleicheniaceae, and the same line of interpretation can be applied to the Schizaceae, where the elaboration is in many respects parallel to the Ophioglossaceae. The distinction is also

<sup>1</sup> Handwörterbuch der Naturwissenschaften (1913), Bd. iii, p. 935. The full statement of the alternative view of a relationship to the Lycopodiales is in the Land Flora (1908).

suggested in the case of the Marattiaceae when the young plants are considered. The most interesting comparisons are, however, with the extinct Zygopterideae and Botryopterideae, and with the Osmundaceae, which have been shown by recent investigation to show signs of a common origin with these. It would lead too far to follow the comparisons in detail at present, but it may be pointed out that the closest comparisons can be drawn between *Helminthostachys* and those forms in which the inner xylem consisted of elongated tracheides mixed with parenchyma, rather than with those in which a gradual conversion of actual elements of the inner xylem into short parenchymatous cells is suggested. The two processes are, however, probably not fundamentally distinct. *Osmunda* appears to still possess a zone of inner xylem around its pith, and is thus comparable with *Helminthostachys*. The parallel is even closer between *Helminthostachys* and some Zygopterideae, notably *Metaclepsydropsis duplex*, *Ankyropteris Grayi*, and *A. corrugata*, although a central pith is not present in them. The steles of these plants with mesarch decurrent protoxylems are, in fact, paralleled by the condition found in the smaller stems of *Helminthostachys* with a mixed pith, the inner tracheides being histologically different from those of the outer xylem. The condition in *Helminthostachys* with a mesarch but solid xylem may be compared with what is sometimes found in *Botryopteris*.<sup>1</sup> Whether the comparison can be extended to some other Botryopterideae and to the Hymenophyllaceae, where the narrow inner elements of xylem appear, on our present knowledge, to be really protoxylem, is not so clear. The protoxylem may possibly be central in some very slender young plants of *Helminthostachys* where the inner metaxylem is practically wanting, but in all strong plants the metaxylem was mesarch as described.

The parallel may be extended to the primary structure of some Cycadofilices, where the inner xylem is of the *Heterangium* type, and outer primary xylem is seen in the leaf-traces. The significance of smaller remains of centripetal xylem, as in *Lyginodendron*, will be referred to below in connexion with the leaf-trace structure.

(b) Secondary thickening. In *Helminthostachys* there is normally no secondary growth, though (as is also seen in the Zygopterideae and some other Ferns) there may be an approach to an irregular radial serration of the tracheides. As is shown by the pieces of rhizome which bore branches, however, a localized development of tracheides in the normal position of secondary xylem can take place long after the primary structure was mature. Though there is no regular persistent cambium, this must be regarded as a special case of secondary thickening, all the more interesting because something is known of the stimulus that starts the process. It may, on the one hand, be compared and contrasted with the more regular secondary thickening in *Botrychium*, and, on the other, with the secondary

<sup>1</sup> Cf. Benson, Ann. of Bot., xxv, Pl. LXXXII, Fig. 13 A.

thickening known in the stems of some Zygoterideae. The marked secondary thickening in *Botrychioxylon* appears more closely comparable to what occurs in *Botrychium*, but the stem of *Ankyropteris corrugata* appears to offer features suggesting a comparison with *Helminthostachys*. It may not be without significance that the only specimen of *A. corrugata* in which secondary thickening has been described and figured was branching. The secondary growth, described as 'only a local formation superadded to the normal woody zone',<sup>1</sup> is most marked in the larger of the two steles shown in the cross-section of the branching specimen. The few existing Ophioglossaceae afford examples of all grades of replacement of the primary wood, with both centripetal and centrifugal xylem, by the secondary wood, and raise the same difficulties in homology and nomenclature as are met with in the case of Cycadofilices and Gymnosperms.

(c) The peculiarities of the departing leaf-trace of *Helminthostachys* afford data for comparison with long-extinct plants, which are remarkable features to find in an existing plant with no known fossil history. These features make it easier to compare the leaf-trace of *Helminthostachys* with the Zygoterideae and such Cycadofilices as *Calamopitys* and *Lyginodendron* than with other existing Ferns, except perhaps the Osmundaceae. No detailed comparisons will be made here, and this is the less necessary since the main points to which reference must be made are raised in recent works on the Zygoterideae, and were summarized by Dr. Paul Bertrand in the 'Progressus Rei Botanicae' for 1912.

It must be borne in mind that while the leaf-trace as it arises from the stele is monarch in the Ophioglossaceae, Osmundaceae, and the Cycadofilices mentioned, it already possesses two groups of protoxylem where it separates from the stele in those Zygoterideae in which the leaf-trace departure is best known. This is not an essential distinction, since the two protoxylem poles in the Zygoterideae may be regarded in some cases at least as derived by division of one, and since further there is some reason to regard the traces of the simplest known Zygoterid trace (*Clepsydropsis*) as having been monarch at departure. It is a question of how far down into the stem the double nature of the leaf-trace is continued.

Dr. Paul Bertrand and Kidston and Gwynne-Vaughan agree in comparing a stage in which the departing leaf-trace of such an Osmundaceous plant as *Thamnopteris* exhibits two protoxylem groups immersed in the metaxylem of the trace, with the structure exhibited by the petiolar bundle of *Clepsydropsis*. The traces of *Asterochlaena*, *Metaclepsydropsis*, and *Diplolabis* pass through a similar stage. Those of *Ankyropteris Grayi* and *A. corrugata* only differ in having a small central extension of the inner xylem or mixed pith of the stele. This soon dies out, and the central portion of the trace xylem becomes solid. This clepsydroid stage is of

<sup>1</sup> Scott: Trans. Linn. Soc., vii, 1912, p. 376, Pl. XL, Fig. 19.

importance as affording a simple expression of the double leaf-trace which assumes such complicated forms in the petioles of the Zygopterideae. If the origin of the trace of *Clepsydropsis* itself, as described by Bertrand, be included in the comparison, we can contemplate a preceding stage in which the xylem of the departing trace includes a single protoxylem. In any case this monarch stage is realized in *Thamnopteris*, and may be spoken of as the pre-clepsydroid stage.

Dr. Bertrand's interpretation of these structures differs considerably from that of Kidston and Gwynne-Vaughan.<sup>1</sup> The essential point for our purpose is brought out by the nomenclature used by the former investigator, who describes all these traces as 'divergeants fermés'. This idea of a closed divergent appears to imply that the outer xylem of the trace is completed adaxially. It may or may not include a portion of the mixed pith or inner xylem, but except in so far as it does this it is not mesarch in the sense in which this term applies to the stelar tube of *Helminthostachys*, i. e. as being composed of outer and inner xylem. This conception of a closed divergent plays an important part in the detailed accounts of Fern anatomy by Bertrand and Cornaille, and has recently been more directly applied to the Cycadofilices by Chodat. The clepsydroid stage is on this view naturally regarded as composed of two closed divergents.

While the other Ophioglossaceae<sup>2</sup> have leaf-traces that are most readily compared with the C-shaped trace of many recent Ferns, *Helminthostachys* in the variety of its leaf-trace departure comes into line with the plants referred to above. As has been shown in the detailed descriptive account, its leaf-trace exhibits every degree of adaxial completion of the arc of outer xylem with which it is continuous in the stem. It goes far to justify the conception of the closed divergent as distinct from the mesarchy exhibited by the stelar tube of xylem. The inner xylem may or may not extend for some distance into the departing trace, being included within the closed divergents of the pre-clepsydroid or clepsydroid stages. As in the case of *Ankyropteris*, the inner xylem soon disappears from the trace, but its presence enables a clear distinction to be drawn between the adaxial completion of the xylem of the trace and true mesarchy. There is a striking resemblance between the clepsydroid stage of the departing trace in *Helminthostachys* and that in *Clepsydropsis* and the various Zygopterideae mentioned above. The pre-clepsydroid stage in *Helminthostachys* is comparable to the similar monarch stage in *Thamnopteris*, especially when the adaxial completion of the xylem takes place before separation from the stelar xylem.

<sup>1</sup> P. Bertrand: *Progressus Rei Botan.*, vol. iv, p. 182; Kidston and Gwynne-Vaughan: *Proc. R. S. Edinb.*, vol. xxviii, p. 433, and *Trans. R. S. Edinb.*, vol. xlvi, p. 654, and vol. xlvii, pp. 469 ff.

<sup>2</sup> The occasional adaxial completion of the xylem in *Botrychium lunaria* must be remembered in this connexion. *Ann. of Bot.*, xxvii, pp. 223 and 239.

It would lead too far to extend the comparison in detail to the Cycadofilices and the Cycadaceae, but it must be pointed out that there is a close resemblance between the monarch stage of the leaf-trace in *Helminthostachys* and in *Calamopitys*, a resemblance extending to the stage of division of the trace in the cortex. This comparison may be also applied to *Lyginodendron*, and the reality of the adaxial completion of the xylem in *Helminthostachys* supports the interpretation of the 'mesarch' bundles of *Lyginodendron* as closed divergents.<sup>1</sup> This view does not necessarily prevent a comparison of the bundles of the latter plant with those of Cycads, for one way of regarding them would be as derived from such a closed divergent.

These comparisons, without fully treating the subject, will indicate that the leaf-trace of *Helminthostachys* resembles those of the Osmundaceae, Zygopterideae, and Cycadofilices more than it does those of the more modern leptosporangiate Ferns.

(d) The branching of *Helminthostachys* also affords suggestive points of comparison with the Zygopterideae and Cycadofilices. The branching is definitely axillary, and buds or branches in this position are known in some Zygopterideae and in *Lyginodendron*. Among other Ferns axillary branching is only known in the Hymenophyllaceae. This position of the lateral branches is the characteristic one in the Cordaitales and in the modern Conifers and Angiosperms.

The branching of *Helminthostachys* may first be compared with that of *Botrychium*. In both these genera of Ophioglossaceae there is a regular development of dormant lateral growing points or vestigial buds in an axillary position. From these, when the normal correlation is disturbed by the arrest of the growth of the main axis, lateral branches may develop. In *Botrychium lunaria* the vascular system of the branch is in relation with the adaxial side of the subtending leaf-trace. In *Helminthostachys*, on the other hand, while the relative position of the branch and the subtending leaf-trace is the same, the vascular connexion is with the stele of the rhizome some distance above the level of the leaf-trace departure. In the case of one branch at least it has been proved that the inner xylem of the branch stele was continuous with the inner xylem of the rhizome, while the outer xylem was continuous with an accessory or secondary development of the outer xylem of the main stele. Had the branch developed at once, instead of after the stele of the rhizome was mature, the inner and outer xylems would both doubtless have been continuous from the main stele to that of the branch. This primary relation is indicated in the structure of the vascular disturbance behind some vestigial buds.

The vascular connexion of the axillary branch in *Botrychium lunaria*

<sup>1</sup> Chodat: Arch. d. Sc. Phys. et Nat., 1908. Cf. also P. Bertrand: Études sur la Fronde des Zygopteridées (Lille, 1909), pp. 264 ff.

is comparable to what is found in the Hymenophyllaceae and in some Zygopterideae, such as *Ankyropteris Grayi*. On the other hand, the relation of the branch-stele in *Helminthostachys* to the stele of the main axis, and not to that of the subtending leaf-trace, is comparable to what is found in some other Zygopterideae, where, however, a relation of the branching to a leaf-axil is not established. Thus the branching in *Botrychioxylon*, *Ankyropteris corrugata*, *Diplolabis Römeri*, and *Metaclepsydropsis duplex* is described as dichotomous, 'using the word in the somewhat loose sense, which is inevitable when dealing with fossils'. The facts regarding the branching of the Zygopterideae have been recently summarized and discussed by Scott, and it is only necessary to refer to his recent papers.<sup>1</sup> It is clear that in this group of plants we meet both with branches standing in the leaf-axils and connected with the meristele of the subtending leaf, and with branches the steles of which were related directly to the stem. In the latter cases, to which the term dichotomy is applied, the two branches were sometimes equal, and in other cases the appearance was of a smaller stele attached laterally to a main one. Dr. Scott leaves it open whether the dichotomy is the more primitive condition, the axillary association with leaves being secondary and derived, or whether the dichotomies may be really cases of modified lateral branching. The regular presence of dormant axillary apices in *Botrychium* and *Helminthostachys* appears to be in favour of the relation between leaf and branch being part of the primary construction of these plants. The vascular relations of the branches in *Helminthostachys* are directly with the stele of the rhizome. It is easy to see how with greater separation of the branch from its subtending leaf, and somewhat stronger development of the branch, a state of affairs would result indistinguishable from what is described as dichotomy in some Zygopterideae. Were a branch rudiment to develop close to the growing point of the rhizome of *Helminthostachys*, the main rhizome continuing its growth, an appearance of equal dichotomy would probably result.

In any case there is a striking parallel both in the variety in the vascular relations of the branches to the main shoot, and in the details of the vascular connexions, between the Zygopterideae and the Ophioglossaceae. The regular presence of lateral branches or suppressed buds in some of these plants must be given due weight in considering the general question of whether lateral monopodial branching is primitive or derived from dichotomy. The case of the Ophioglossaceae appears to support the former view.

The argument for a relationship of Ophioglossaceae with Zygopterideae is clearly stated and advocated from the side of the extinct plants in Dr. Scott's paper on *Botrychioxylon*. More detailed study of *Helminthostachys* strengthens the case for this affinity from the side of the existing

<sup>1</sup> Ann. of Bot., xxvi (1912), pp. 57-60; Trans. Linn. Soc., 2nd ser., Bot. vii.

plants by revealing additional and unsuspected resemblances in the morphology and anatomy of the stem and leaf-trace. If our knowledge of the anatomy of *Helminthostachys* was based on the four pieces of rhizome illustrated in Text-figs. 2, 3, Text-fig. 4, Text-figs. 5 and 6, and Text-fig. 8, it may reasonably be stated that we should find our closest comparisons not among existing Ferns, but with the palaeozoic Zygopterideae and Cycadofilices. If these resemblances indicate the true affinities of the isolated group of the Ophioglossaceae, the latter present an interesting contrast, as regards the evidence, to the Osmundaceae, which there is also reason to trace back to the ancient Fern stock. In the case of the Osmundaceae we have fossil remains extending our knowledge of the group back to Permian times. In the Ophioglossaceae the conclusion that there is a true affinity with Zygopterideae seems, if anything, more inevitable in the light of the primary structure of the stele, the secondary thickening, the structure of the leaf-trace, and the nature of the branching. The evidence is here derived from comparative anatomy with a practical absence of any historical record between the early palaeozoic Ferns and the Ophioglossaceae of the present day.

#### SUMMARY.

1. The rhizome of *Helminthostachys* exhibits a general segmental construction, and may be regarded as composed of three series of segments, one ventral and two dorso-lateral. The insertion of a leaf, with its stipular sheath and axillary bud, corresponds to each dorso-lateral segment, the leaves alternating right and left of the median line. The ventral segments do not bear leaves. This arrangement holds throughout the plant.

The transition from a leaf into its region of the stem is a gradual one, and it is hardly possible to speak of definite internodes in the adult plant. In the slender shoots of young plants the leaf insertions are more separate and internodes may be distinguished, at least in the stelar anatomy.

It is not stated that the segmental construction of the rhizome is related to the cell-segmentation at the apex, though it would be consistent with such an explanation.

2. The stele of the adult rhizome has a large pith, and the tube of xylem around this is mesarch, i. e. consists of outer and inner xylem, the protoxylem elements being found between them. The inner xylem may be more or less extensively represented; in smaller rhizomes there may only be scattered tracheides at the periphery of the pith, and sometimes the latter abuts directly on the protoxylem.

3. The roots are endogenous, but only penetrate a comparatively thin zone of cortical tissue. The xylem of the root is continuous with the outer xylem of the stele of the rhizome, and when the inner xylem of the latter



is poorly developed there may be a more or less direct continuity of the parenchymatous pith of the root and rhizome. The pith in the root base sometimes has a well-marked internal endodermis independent of that often found in the rhizome.

4. The dorsal side of the stele is disturbed by the leaf-traces and in relation to the vestigial buds.

The leaf-trace exhibits considerable variety in its mode of departure, structure, and mode of division in the cortex. In large rhizomes it may depart as a mesarch portion of the stelar tube, though the inner xylem is always less developed within the trace, and gradually dies out as the latter departs. In other cases the inner xylem disappears from the nascent leaf-trace, which departs with an endarch xylem.

The leaf-trace, whether mesarch or endarch, may divide into two in the cortex without adaxial completion of the outer xylem or the other tissues. More usually, this adaxial completion of the xylem takes place before division, and the phloem as well as the endodermis may also be completed. Adaxial completion may take place just before division of the trace; in other cases the xylem is completed adaxially while the trace is still monarch, and sometimes even before its xylem is free from that of the stele. This condition, in which the monarch trace has a complete ring of outer metaxylem surrounded by phloem and endodermis, may persist until the undivided trace leaves the rhizome. Usually, however, division of the trace is effected, and just before this the leaf-trace has a dumb-bell-shaped outline with a protoxylem group in each half, the metaxylem being continuous from the abaxial to the adaxial xylem between the two protoxylem groups. This has been termed (following C. E. Bertrand and Cornaille) the clepsydroid stage.

In leaf-traces which are mesarch and also show adaxial completion of the xylem, a clear distinction can be drawn between the two conditions; they both involve the presence of metaxylem to the inside and outside of the protoxylem, but are essentially different. The leaf-traces of small rhizomes are always endarch at departure, and may or may not exhibit more or less complete adaxial extension of the xylem.

5. The disturbance of the stele in relation to the vestigial axillary buds varies in degree, being most marked in the case of large adult rhizomes, and wanting in many smaller ones. In all cases, however, the endodermis remains open, or opens again, in relation to the dormant lateral apex. In well-marked examples, the bulge of xylem behind the bud corresponds to the stele at the base of a lateral branch in consisting of inner xylem surrounded by outer xylem, both being continuous with the corresponding tissue of the main stele.

The vestigial bud is in relation to the subtending leaf, and exhibits some variety in position relatively to the corresponding leaf-gap; it is



usually at the level of the separation of the next leaf-trace, but is sometimes further forwards and in smaller rhizomes nearly always further back.

6. The vascular relations of two branches, developed from lateral buds, to their parent rhizomes are described, and place the nature of the dormant buds beyond doubt.

The xylem of the stele of the branch is composed of a central strand of tracheides of the inner xylem surrounded by a zone of outer xylem. The inner xylem is continuous with the corresponding tissue of the main stele, while the outer xylem of the branch is continuous with the outer xylem of the rhizome, or rather with a secondary development of this, to which the name 'accessory xylem' has been given.

This accessory xylem has the characters of an irregular secondary thickening of the stele of the rhizome, and may be developed not merely in the direct tract backwards of the branch stele, but all round the main stele and before and after the branch departs.

7. The inner xylem is unequally developed at different levels in the adult rhizome, and exhibits a regular rhythm of decrease and increase in the dorsal region of the stele. It diminishes or disappears opposite the nascent leaf-trace, and is especially strongly developed as the leaf-gap closes.

A corresponding rhythm is traceable in slender juvenile rhizomes. The inner xylem may be largely replaced by parenchyma before a leaf-trace begins to separate, but increases again in amount as this happens, bridging across the gap left in the outer xylem by the departing trace. In small rhizomes this increased development of the inner xylem may lead to the stele becoming solid on the departure of a leaf-trace, to become medullated again as the next trace is initiated.

8. The structure of slender rhizomes with the juvenile type of stele is described both for plants developed from the embryo and for branches. These show essential agreement, with differences in detail depending partly on the strength of the shoot, and partly on the earlier or later development of a pith by replacement of most of the inner xylem by parenchyma.

The inner xylem in the young plant and the slender basal region of the branches consists of spirally thickened tracheides, and histologically resembles protoxylem. Its real nature is shown when the true protoxylem is distinguishable between outer and inner xylem in relation to a nascent leaf-trace, and by the behaviour of the inner xylem at the separation of the trace. That the more central xylem of the branch, in spite of its histological characters, is to be regarded as inner xylem is further shown by its continuity with the inner xylem of the main axis.

9. The juvenile type of anatomy may be maintained for many nodes, and no example showing the transition from the seedling structure to the adult type has been studied. Rhizomes of different sizes show, however, that the change from the juvenile type of structure to the adult condition

(in which the stele has a large and definite pith and a well-developed inner xylem consisting of pitted tracheides) is to be regarded as a process of further expression or elaboration of the structure found in the more slender rhizomes. It is probably related to increased strength and nutrition of the plant.

An exceptionally rapid transition from a condition with a solid stele to the fully adult type is described in what was probably a large well-nourished branch.

10. That the juvenile type of structure is to be regarded as an expression in miniature of what is found in full-sized rhizomes, and is to be explained on physiological grounds, rather than as a necessary phylogenetic recapitulation in the development of the individual, is further shown by full-sized rhizomes which become more and more slender. In such cases, presumably dependent on poorer nutrition, the stele with adult structure assumes in the slender continuation of the rhizome the juvenile size and structure. A condensation of structure is shown in such cases, the converse of the expansion or elaboration exhibited in the normal ontogeny.

11. The segmental construction of the rhizome, referred to in the first paragraph of the summary, is more or less clearly reflected in the stelar anatomy. Corresponding to the ventral segments, a lower, purely cauline portion of the stele is recognized. By tracing the tissues towards the apex this is confirmed. The stele is regarded as in part at least cauline, and not as made up of the united leaf-traces enclosing between them a portion of the ground tissue as the pith, as on Campbell's interpretation. Both the segmental construction of the shoot and its morphological unity, as expressed in recognizing axis as well as leaves, have to be taken into account.

12. Comparisons are made with the Zygopterideae and Cycadofilices as regards the outer and inner xylems, the secondary thickening, the peculiarities of the leaf-trace, and the nature of the branching, and confirm the general view of a relationship between the Ophioglossaceae and the more primitive Ferns.

## DESCRIPTION OF FIGURES IN PLATES I-III.

Illustrating Professor Lang's paper on *Helminthostachys zeylanica*.

(All these figures are from untouched photographs.)

*e.*, endodermis; *e.i.*, internal endodermis; *ph.*, phloem; *x.o.*, outer xylem; *x.i.*, inner xylem; *px.*, protoxylem; *ab.*, abaxial portion of xylem of leaf-trace; *ad.*, adaxial portion of xylem of leaf-trace; *x.<sup>2</sup>*, secondary or accessory xylem.

### PLATE I.

Photo 1. Portion of vascular tube of a rhizome of adult type, showing the well-developed inner xylem.  $\times 67$ .

Photo 2. Similar portion of the vascular tube from a smaller rhizome; the inner xylem is represented by scattered elements.  $\times 67$ .

Photo 3. Similar portion of the vascular tube from a rhizome with irregularly developed inner xylem; in the centre of the portion figured the protoxylem abuts on the parenchyma of the pith.  $\times 67$ .

Photo 4. Transverse section of a large adult rhizome, showing the stele, from which a leaf-trace has departed, leaving the leaf-gap. The leaf-trace is seen in the clepsydroid stage in the cortex. The basal connexions of two roots with the stele are shown.  $\times 25$ .

Photo 5. Leaf-trace from the adult rhizome figured in Photos 4 and 1, and Plate II, Photos 20, 21, showing an advanced stage of the adaxial extension of the xylem. The protoxylem of the trace has divided in preparation for the division of the trace; internal to each of the two groups of protoxylem, a small amount of inner xylem persists.  $\times 67$ .

Photo 6. Same leaf-trace as in the preceding photograph, but a little further out (cf. Photo 4). The outer xylem is complete adaxially, and a band of tracheides connects the abaxial and adaxial portions, dividing the central parenchyma into two portions. In each of them remains of the inner xylem are still present in front of the protoxylem groups.  $\times 67$ .

Photo 7. Dorsal portion of a stele of a small rhizome, showing to the right the disturbance of the endodermis in relation to a vestigial bud and on the left the arc of xylem for a leaf-trace; the inner xylem is almost wanting within the protoxylem of the trace.  $\times 67$ .

Photos 8, 9. Two stages in division of the leaf-trace seen in Photo 7. The division takes place without adaxial completion of the xylem, though a number of metaxylem elements are present internal to the protoxylem groups.  $\times 67$ .

Photo 10. Transverse section of a leaf-trace from a small rhizome (that in Text-fig. 4), showing endarch xylem with no adaxial completion of the outer xylem.  $\times 67$ .

Photo 11. Transverse section of the leaf-trace shown departing from the cortex of the large rhizome in Text-fig. 6. The trace is monarch, the xylem has closed round to form a complete tube, and the protoxylem is separated from both the adaxial and abaxial regions of the xylem by parenchyma.  $\times 67$ .

Photo 12. Transverse section of another leaf-trace from the same rhizome, showing the monarch trace, the absence of inner xylem, and the adaxial extension of the outer xylem still incomplete.  $\times 67$ .

Photo 13. The same trace as in Photo 12, a little further out. The adaxial extension of the xylem is complete, and the phloem also forms a complete tube.  $\times 67$ .

Photo 14. Dorsal portion of the stele of the rhizome in Text-fig. 8, showing the preparation for the departure of the third leaf-trace. The adaxial extension of the outer xylem is happening as the arc of xylem separates.  $\times 67$ .

Photo 15. Further stage than Photo 14. The adaxial xylem is complete, but is still continuous with the xylem of the stele.  $\times 67$ .

Photo 16. The same leaf-trace in the cortex, showing the complete tube of phloem. The xylem of the trace is still monarch.  $\times 67$ .

Photo 17. The same leaf-trace in the clepsydroid stage.  $\times 67$ .

Photo 18. Similar but larger leaf-trace from the same rhizome in the clepsydroid stage.  $\times 67$ .

Photo 19. One of the halves into which this trace divided (cf. Text-fig. 8, E), showing the preparation for the next division.  $\times 67$ .

## PLATE II.

Photo 20. Dorsal portion of the stele of the rhizome represented in Text-fig. 2, C. To the left the nascent leaf-trace is seen with the inner xylem diminished in amount but not wanting. To the right is the closed leaf-gap with the greatly developed inner xylem continuous across and extending outwards into the gap still present in the outer xylem. Above this the endodermis is raised and open in relation to the vestigial bud.  $\times 67$ .

Photo 21. Similar section, corresponding to Text-Fig. 2, D, showing the full development of the bulge of xylem in relation to a vestigial bud. The bulge consists of inner xylem covered by outer xylem, while the outer xylem is also extending across below to complete the xylem ring of the main stele.  $\times 67$ .

Photos 22-24. Stages in the departure of the vascular supply from the first branching specimen.

(Cf. Text-fig. 4.) In Photo 22 the endarch leaf-trace is departing, its protoxylem being seen at *px*. *x*.<sup>2</sup> indicates one of the two groups of accessory xylem, while *x*.*i* points to the inner xylem extending into the leaf-gap and destined for the branch. In Photo 23 the inner xylem has passed through the gap and approximated to the uniting groups of accessory xylem. In Photo 24 the stele of the branch is fully constituted and lies beside the broken-down stele of the parent fragment of rhizome.  $\times 67$ .

Photo 25. Transverse section of the stele of the second branching specimen after the branch had passed off. The stele shows outer and inner primary xylem, and outside the former accessory or secondary xylem is present all round the stele.  $\times 25$ .

Photo 26. Portion of a similar section more highly magnified, showing the relations of the accessory or secondary xylem to the primary xylem.  $\times 67$ .

Photos 27-29. Stages in the departure of the vascular supply from the second branching specimen. (Cf. Text-fig. 6.)  $\times 67$ . Photo 27 shows the great development of accessory or secondary xylem to the sides of the closed leaf-gap. It is uncertain whether the xylem marked ? is to be regarded as inner xylem passed into the gap, as in the first branching specimen. Photo 28 shows the arc of accessory xylem destined to form the outer xylem of the branch completed over the closed gap. Extending into it are the narrow tracheides of the inner xylem mixed with parenchyma. Photo 29 shows the basal region of the stele of the branch in longitudinal section, and the continuity of its outer and inner xylem respectively with the tissues recognized in Photo 28.

#### PLATE III.

Photos 30-34. Stele of the first branch in transverse section, showing the preparation for the separation of the first leaf-trace and the position of its protoxylem (Photo 30); a later stage on the separation of the xylem of the first leaf-trace (Photo 31); the first leaf-trace, with its xylem completed adaxially, lying beside the xylem of the stele of the branch, which has now regained its complete structure (Photo 32); the stele of the branch after departure of the first leaf-trace and at the level of the vestigial bud (Photo 33); and a later stage in the preparation for departure of the second leaf-trace, the protoxylem of which was evident in the preceding photograph (Photo 34).  $\times 67$ .

Photo 35. Longitudinal section of the basal portion of the second branch, showing the departure of the leaf-trace, which leaves a gap in the outer xylem; the inner xylem is continuous along the gap and separates the pith from the parenchyma in the angle of the departing trace. The inner xylem consists of spiral tracheides, while those of the outer xylem are pitted.  $\times 67$ .

Photos 36-39. Stele of the second branch in transverse section, showing the stages in initiation and departure of the second leaf-trace. In Photo 36 the first leaf-trace is seen divided in the cortex, the stele is medullated, and the protoxylem of the second leaf-trace is recognizable in its xylem; the inner xylem is almost entirely replaced by pith. In Photo 37 the endarch xylem of the second leaf-trace has separated, and elements of inner xylem have developed below the gap left in the tube of outer xylem. In Photo 38 this development of inner xylem is still more marked, and inner xylem is seen in the lower part of the stele, where a root is attached. In Photo 39 the leaf-trace is separate and has its complete endodermis; the inner xylem of the stele, which has returned to the condition shown in Photo 36, has largely disappeared, and the protoxylem of the third leaf-trace is evident in the xylem tube.  $\times 67$ .

Photos 40-46. Transverse sections of the stele of a young plant developed from an embryo, showing the changes involved in the separation of the first and second leaf-traces. Photo 40 shows the stele low down in the hypocotyl, with a central group of inner xylem surrounded by an irregular zone of outer xylem. Photo 41 shows the group of parenchyma cells internal to the arc of outer xylem destined for the first leaf-trace; the protoxylem of this is endarch. In Photo 42 the first trace has separated, and the xylem of the stele has again become solid. In Photo 43 most of its inner tracheides are replaced by parenchyma, while the second leaf-trace is initiated as an endarch arc of the outer xylem. Photo 44: the trace is not separate, but the increased development of inner xylem in the stem stele has filled up the gap to be formed in the outer xylem when separation takes place, and rendered the stem stele almost solid. Photo 45 shows a slightly further stage, the trace xylem not being completely free, while the outer xylem of the stem stele is reconstituted around the central group of inner xylem. In Photo 46 pith is again appearing by the replacement of most of the inner tracheides by parenchyma; the protoxylem of the third leaf-trace is evident.  $\times 130$ .

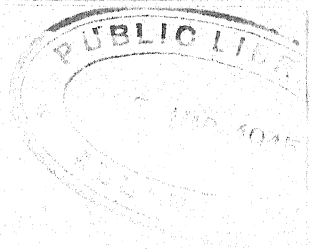
Photos 47-49. Separation of a leaf-trace from a stele of a small medullated rhizome of adult type. The trace is endarch at origin (Photo 47); the outer xylem becomes completed adaxially, while the trace is still monarch (Photo 48); and the trace later passes through the clepsydroid stage (Photo 49).  $\times 67$ .

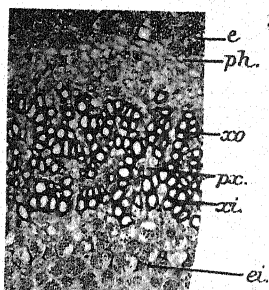
Photo 50. Stele of the same rhizome as Photo 48, at a level nearer to the apex, showing the condensation in size and structure.  $\times 67$ .

Photo 51. Stele of a small adult rhizome, showing a departing leaf-trace, the xylem of which becomes adaxially completed before separation from that of the stele.  $\times 67$ .

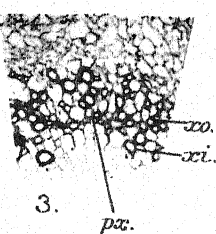
Photo 52. Stele of the same rhizome as that in Photo 51, but nearer to the apex, photographed to the same scale as Photo 51. The stele exhibits condensation or reduction to a purely juvenile type, with scattered tracheides of inner xylem in the undefined pith.  $\times 67$ .

Photo 53. Transverse section of the stele of a young plant somewhat behind the apex. The outline of the stele is evident in the procambial condition, and the first-formed tracheides are seen to occur both dorsally (*lt.*) in relation to the youngest leaf-trace, and ventrally (*px.v.*) in relation to the lower purely cauline portion of the stele.  $\times 67$ .

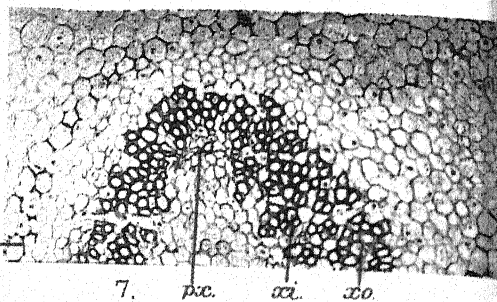




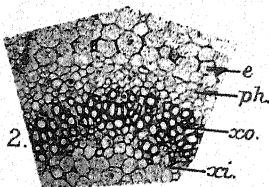
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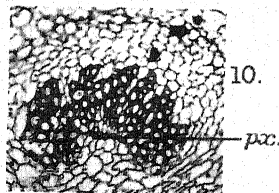
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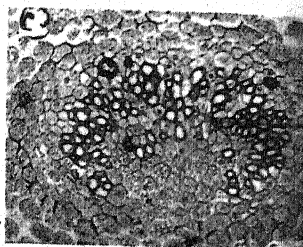
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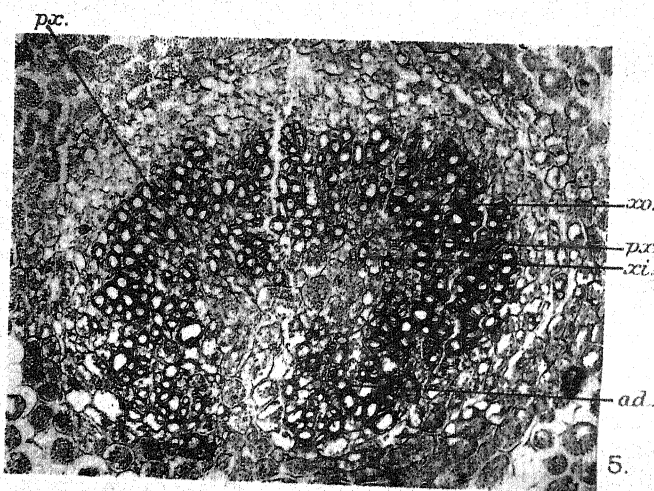
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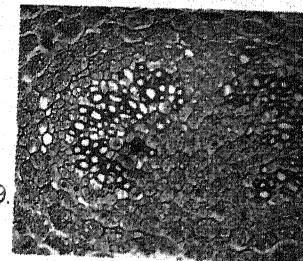
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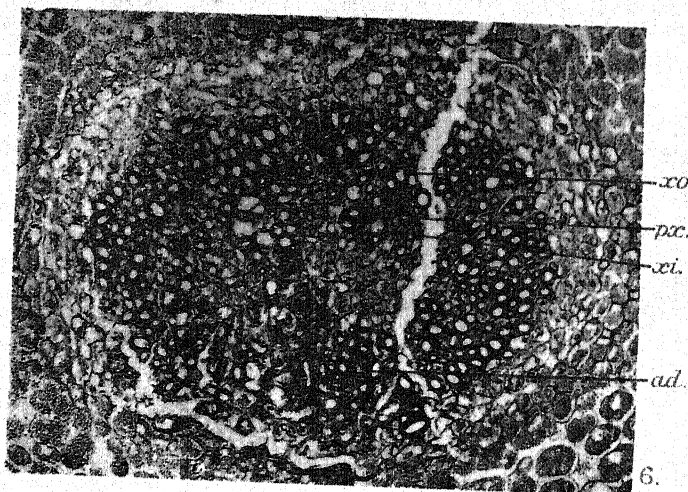
8.



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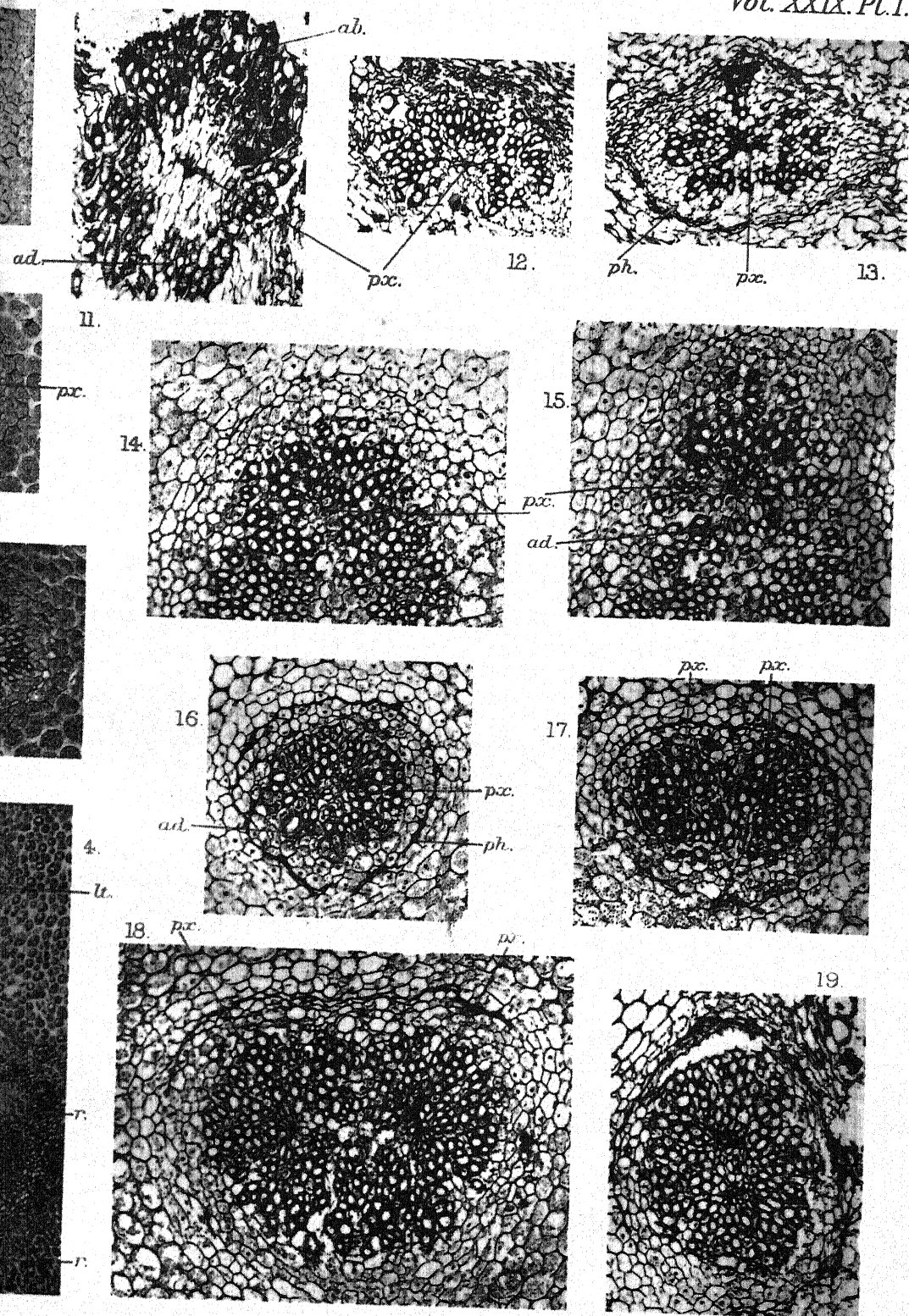
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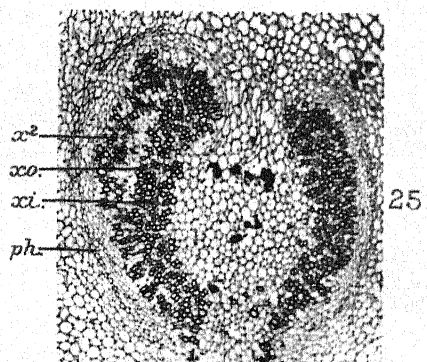
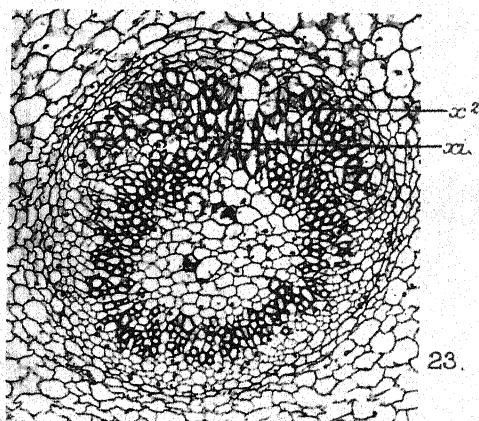
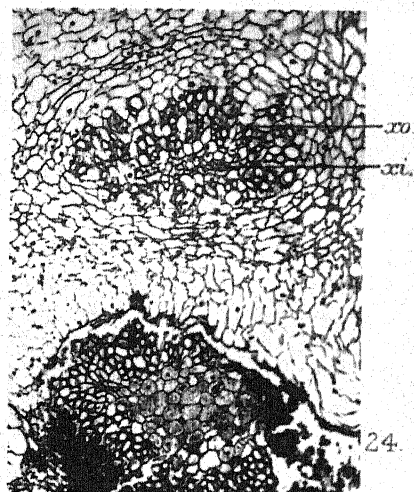
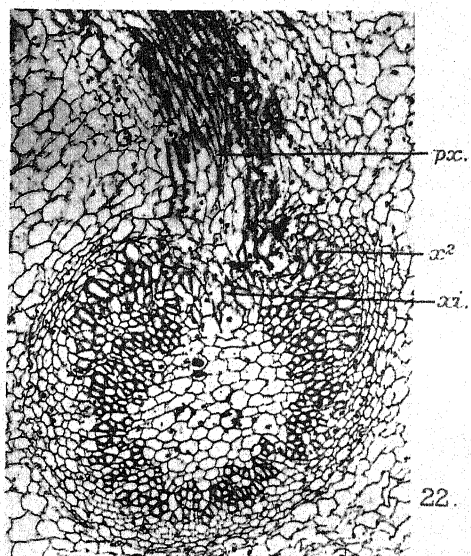
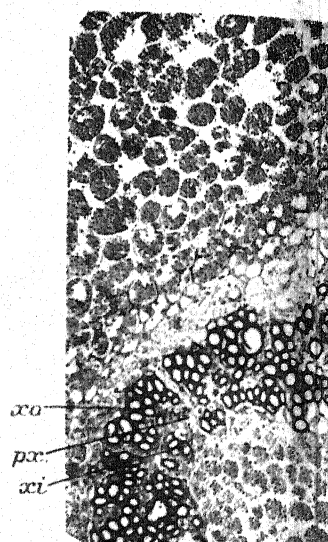
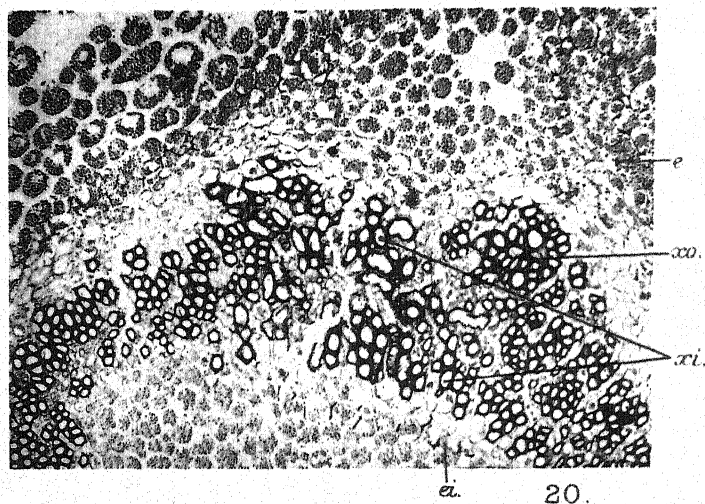
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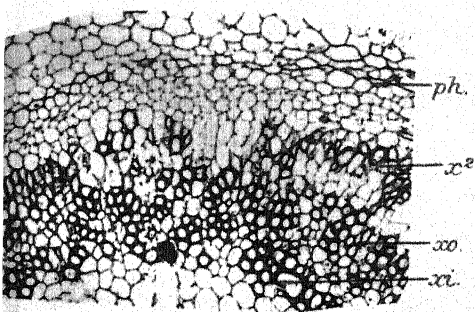
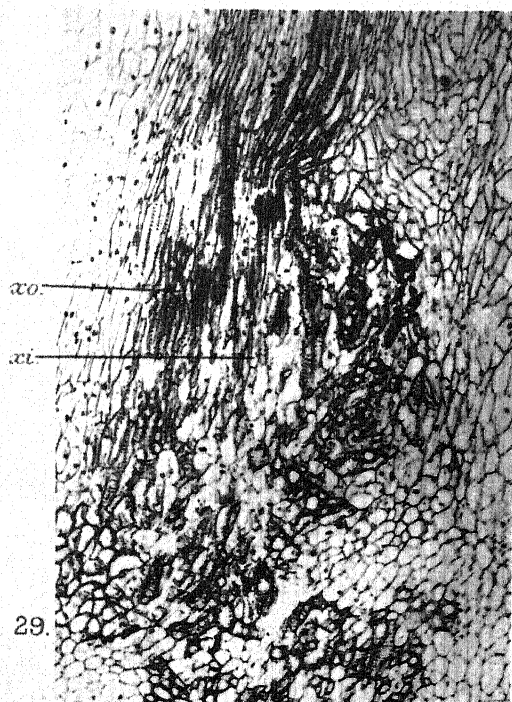
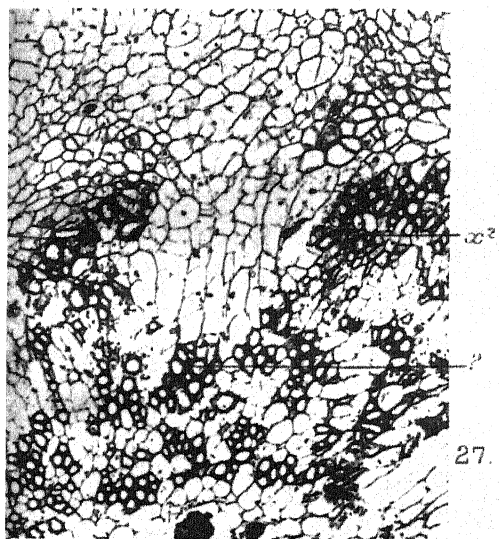
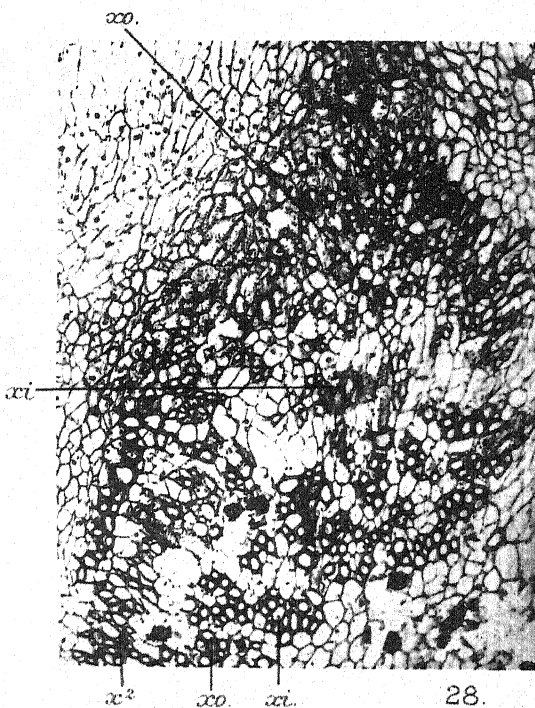
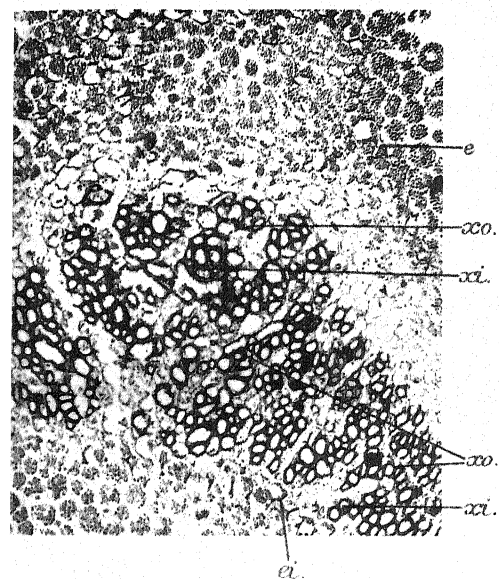




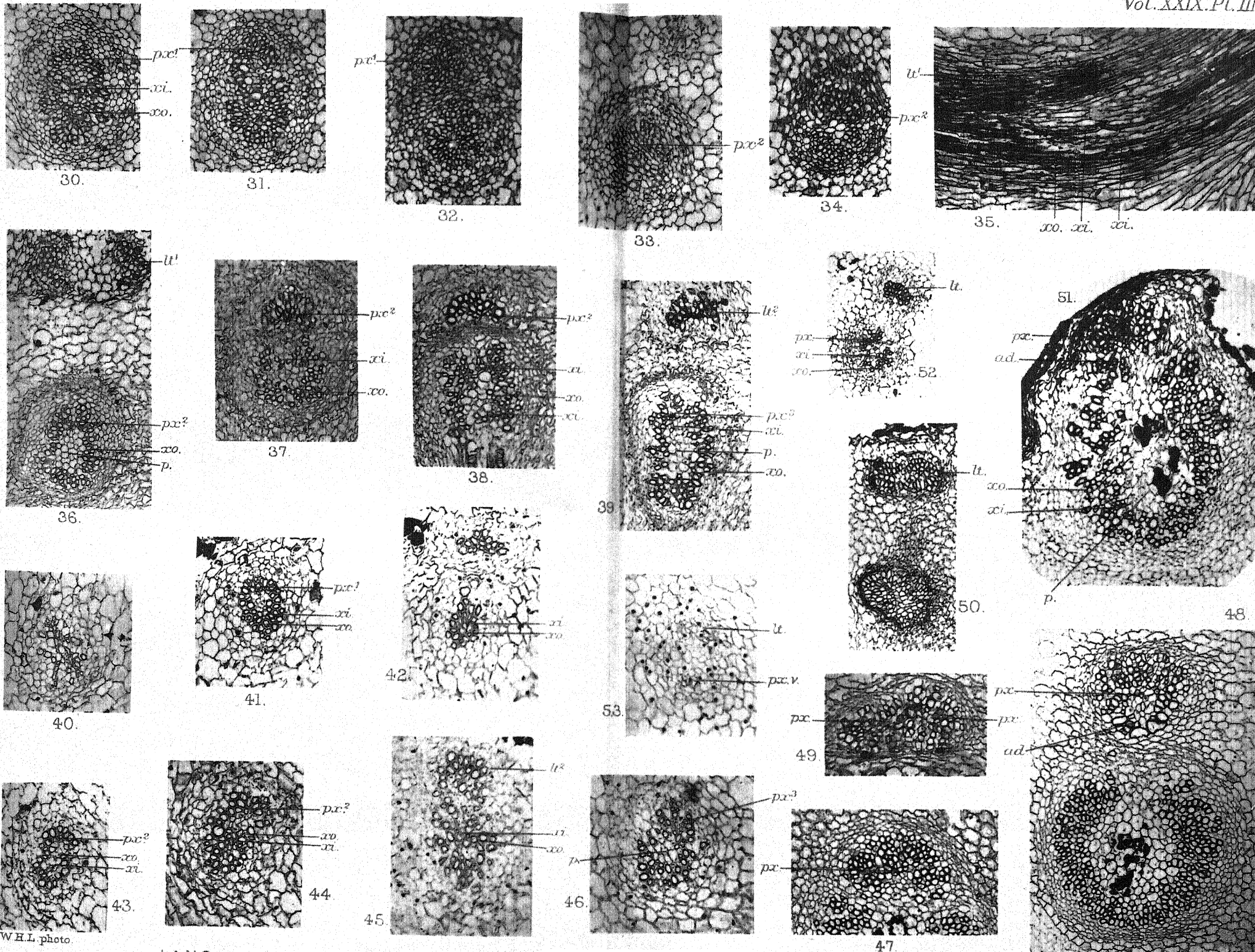


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## A Comparison of the Stem Anatomy of the Cohort Umbelliflorae.

BY

CARL S. HOAR.

With Plates IV and V.

AS classified at present, the families Cornaceae, Araliaceae, and Umbelliferae are placed together under the cohort Umbelliflorae. This cohort is in turn placed at the top of the sub-class Archichlamydeae, next to the sub-class Metachlamydeae. Such is the classification given by Engler and Prantl and followed by Engler and Gilg (1).

The closeness of relationship between the families of this cohort has always been more or less in doubt. Because of this doubt I have made, during the last year, a special study of the stem and root anatomy of the three families. My purpose in so doing has been to learn what additional evidence I might obtain as to their true relationships. In pursuing this study I have concerned myself especially with the Cornaceae, having been able to obtain all of the genera and many of the species native to this region, together with several which are of exotic origin. With the other two families I have had a smaller number of examples, but their constancy of common characteristics seems to confirm my conclusions.

Of the three families, the Cornaceae are generally considered to be the lowest, while the Araliaceae are placed between the former and the Umbelliferae. Such a view might easily be drawn from external characters alone. The Cornaceae are, with one or two exceptions (*Cornus canadensis*), shrubs, which sometimes, as in the case of *Nyssa sylvatica*, become tree-like in appearance. The Araliaceae would seem to stand in an intermediate position, since, although they are often shrubby and even tree-like, yet the wood is seldom as well developed, and many of its members are herbaceous. Finally, the Umbelliferae would seem to be the highest since its members are always herbaceous, with very little wood. It has been conclusively shown that herbaceous plants are derived from the woody plants, frequently by the formation of large rays and the decrease of the amount of woody elements (2, 2 a). For this reason the external appearance of the three families gives the basis for the conclusions which are shown to be true when the flower and stem anatomy are taken into account.

The family Cornaceae is composed, according to Engler and Gilg's classification, of three sub-families, namely, the Mastixioideae, the Curtisioideae, and the Cornoideae. From this family they separate the sub-families Nyssoideae, Davidioideae, and Alangioideae, placing them under the order Myrtiflorae. As I shall attempt to show later, the evidence for such a separation is lacking when the anatomy is taken as the criterion.

Of the various genera of the above sub-families, I have been able to secure examples of *Cornus*, *Nyssa*, *Davidia*, *Griselinia*, *Aucuba*, *Corokia*, *Mastixia*, and *Helwingia*. In the case of *Cornus*, *Nyssa*, *Griselinia*, and *Aucuba*, I have had specimens of the roots as well as of the stems.

Regarding the general anatomical characteristics of the Cornaceae we are indebted chiefly to the work of Sertorius (3). It would seem best, perhaps, to insert here some of the more important anatomical characters which he found in his study of the stem axis. The cork arises usually sub-epidermally, though Moller and Weiss state that the phellogen in *Aucuba* and in some species of *Mastixia* arises from the epidermis. Collenchyma appears in the cortex in nearly all cases, though none has been reported for *Mastixia*. Cortical bundles appear only in the case of *Mastixia*, and are due there to the leaf-trace which passes down in the cortex for a short distance before entering the wood. The hard bast, as de Bary (4) has noticed in *Cornus*, is found only in the primary wood, except in *Mastixia*, where it is formed secondarily and stretches irregularly throughout the soft bast, with no definite arrangement. In *Marlea begoniacefolia*, *Helwingia*, and *Aucuba japonica* no hard bast appears at all. Stone cells are quite common throughout the family.

In regard to the woody axis proper, I will speak first of the vessels. According to Sertorius these are nearly always scalariform. In the genera *Alangium*, *Torricellia*, and *Marlea*, however, there is a single pore in the secondary wood. However, the pore is often drawn out, and in the primary wood bars are found. In some of the above instances one or two bars have been found appearing even in the secondary wood. The vessels themselves are usually small and isolated, with a four-sided appearance from a transverse view. The walls, according to Sertorius, have always bordered pits where the vessels come in contact with the parenchyma of the rays, though Solereder (5) finds simple pits where *Griselinia* is in question. The wood parenchyma has only bordered pits in some cases, while in others it may have both kinds or only the simple pits. Septation of tracheides is not a common feature, though Solereder reports it for *Marlea*, and I have also noticed it in *Aucuba japonica* and *Griselinia lucida* root. The rays are in general small, being from one to five cells in width. In our native species they are not very abundant, but in some of the exotic genera, such as *Griselinia*, *Corokia*, &c., the rays are very numerous, and the individual cells are often quite large.

Among the members of the Cornaceae, *Mastixia* stands out as peculiar. I have already mentioned the peculiarity in regard to the secondary hard bast, and in regard also to the presence of cortical bundles. There is yet another peculiarity which is found in no other place in this family, but which is characteristic of the two higher families. This is the presence of secretory canals in the cortex and in the pith. The presence of these canals has caused much discussion concerning the true relationship of this genus. Baillon (6) placed it with the Araliaceae next in kin to the genus *Anthrophyllum*. Later, he reconsidered and changed his opinion. Van Tieghem (8) came, through his work, to the view that it belonged neither to the Cornaceae nor to the Araliaceae, but rather to the Dipterocarpeae. Later, he also changed his opinion. Burck (9) also came to the view that it stands very near to the Dipterocarpeae, though the Simarubeae and the Liquidambeae must also be taken into the reckoning.

Though the above opinions show that *Mastixia* is a much-discussed genus, and that its position is rather unsettled, yet the other characteristics would seem to indicate that it belongs to the Cornaceae, and here it is generally placed. Granting that *Mastixia* is one of the Cornaceae, it is argued that it must serve as a connecting link between that family and the Araliaceae. The basis for this argument is chiefly the presence of secretory canals in pith and in cortex. Such canals are a constant feature in the Araliaceae and in the Umbelliferae. In answer to the above argument it can simply be said that the presence here of secretory canals does not seem to be conclusive evidence, in this case, of a close relationship. We find in the family Hamamelideae that only the Altingeae, with *Liquidambar* as our common example, are characterized by internal secretory canals. Secretory canals also occur in some members of other families, while they are lacking in other members of the same family. Here, evidently, their presence or absence does not change their relationship. Only in such families as the Araliaceae and the Umbelliferae, where they are present in every member, are they evidence of close relationship. In the Cornaceae, they are found only in *Mastixia*. Hence they are not characteristic of the family, and would not seem to me to be of great value in showing relationship where that family is concerned.

Having now spoken of the more important and noticeable anatomical features of the Cornaceae, let us turn briefly to the like features in the Araliaceae and in the Umbelliferae.

In the Araliaceae secretory canals are present throughout the entire family, being present in all parts of the cortex and often also in the pith. Another peculiarity found nowhere among the Cornaceae is the presence of medullary bundles. These are formed in a ring, having collateral structure, and being inversely orientated so that the xylem is towards the outside. I have noticed these in the petiole of *Aralia chinensis* var. *manchuria*,



though they occur nowhere in the main stem. It is generally conceded that the leaf is more conservative than the stem, and hence we might expect that medullary bundles are an ancient character of the Araliaceae. However, they are not found in the Cornaceae, which are considered to be lower in the evolutionary scale.

In regard to the wood of the Araliaceae, we find often very broad rays which, in the herbaceous species, entirely separate the bundles. The vessels have generally simple, elliptical perforations. In two instances (*Gilbertia* and *Fatsia*) a few bars occur showing an indication of a transition to the scalariform type. The end walls of the vessels are more or less inclined to the lateral walls. Their lateral walls when in connexion with parenchyma of the rays commonly have simple pits. As regards the wood prosenchyma, the pits are always simple and we find delicate septations. Such cells often contain starch, and are called 'septate tracheides'.

Finally, in the stem of the Umbelliferae the cortex often contains strands of collenchyma which lie directly under the ribs appearing upon the outside. Sometimes these are lacking or are replaced by a ring of sclerenchyma. The fibro-vascular system is normally in the shape of a ring of bundles, with often medullary bundles in addition. The bundles are usually isolated, though sometimes they are fused together and are continuous. Their rays are usually either uni- or biseriate. The vessels have usually simple perforations, though sometimes the scalariform type is found accompanying them. The pitting, where the vessels are in contact with the parenchyma of the rays, may be simple or bordered. The wood prosenchyma shows both simple and bordered pits. Anomalous structures sometimes occur, such as cortical bundles and an extra-fascicular vascular ring. The pith is usually lost in the internodes, thus leaving the stem hollow.

Having briefly summed up the situation, and having stated the general anatomical characters of the order, I will now describe in some detail the results of my investigation. Past evidence from material worked over in this laboratory seems to indicate that the distribution of parenchyma is one of the most important and reliable anatomical characters found in plant anatomy. In the Gymnosperms we find it either at the end of the year's growth (terminal) or scattered throughout the annual ring (diffuse). In general, among the Dicotyledons the lower families have their parenchyma scattered throughout the annual ring (diffuse), while higher families have it clustered about the vessels (vasicentric). This character has been found to be so constant and of so great value that it has aided much in determining the relationships between families. Thus the Rosaceae, which from a systematic standpoint seem to be close to the Leguminosae, show a different type of parenchyma distribution and hence, apparently, a much more remote relationship than is ordinarily assigned to them.

Thus far in the anatomical study of the Umbelliferae the value of the



distribution of parenchyma in determining relationships seems not to have been realized. Because of this I have paid especial attention to the subject in my investigation.

As stated above, the terminal connexions of the vessels of the Cornaceae are generally scalariform, while those of the Araliaceae and of the Umbelliferae are characteristically simple elliptical or round pores. I have noted the condition of the terminal openings in my study, and have found results which corroborate the above statements. The condition of the terminal connexions between vessels is not as constant as is the distribution of parenchyma, but it may be mentioned here as worthy of notice.

In recording my results I will first speak of the Cornaceae, it being usually considered to be the lowest family of the three. Among the genera of this family the commonest native genus is *Cornus*. Pl. IV, Fig. 1 shows the type as illustrated in a transverse view of the species *Cornus sanguinea*. Here may be plainly seen the heavy-walled tracheides, the few small rays, and the numerous diffuse parenchyma cells. In Pl. IV, Fig. 2 one may see a radial view of the same species, and the same features may be noted. The genus does not always show such heavy walls, nor is the parenchyma always as abundant. However, the relationship as regards the distribution of parenchyma and the character of the end walls of the vessels persists.

In Pl. IV, Fig. 3 we see a transverse view of the stem of *Nyssa sylvatica*. Here the parenchyma is plainly diffuse, and in other ways the wood closely resembles that of *Cornus*. Fig. 4 is a higher power of the same view as that shown by Fig. 3. Fig. 5 is a radial view of the same, and here we can see the even closer resemblance to *Cornus*, the parenchyma being more strikingly diffuse than in the transverse view.

Here I would call attention to the fact that, under Engler and Gilg's classification, *Nyssa* is placed outside the Cornaceae. Certainly the anatomical structure would not appear to warrant this.

Turning from our native forms to those exotic to this region, we find the parenchyma much less abundant. However, in *Davidia involucrata* (Fig. 6) one can plainly see the diffuse parenchyma and the scalariform vessels from the radial view. The number of bars in the vessel seems to be much more numerous than the number in the case of *Cornus*, but even among members of the same genus we find much deviation from the small number. *Davidia* is another genus placed by Engler and Gilg outside the Cornaceae. Clearly this would not seem possible when the internal anatomy is considered.

In some exotic species the parenchyma appears to be entirely lacking. Its absence, however, is a common characteristic in plants living in a climate where the storage of food is not necessary. In most instances I was able to find remains of parenchyma cells, showing their persistent character.

Fig. 7 is that of a radial section of the stem of *Griselinia lucida*.

This has in most instances the parenchyma transformed into substitute fibres. However, the above figure, which is one of several examples, shows clearly the parenchyma as being diffuse. In cases where the root of the same species was in question, a much greater quantity of diffuse parenchyma appeared. Pl. IV, Fig. 8, a transverse section of the root, and Fig. 9, a radial section of the same, show that that root was dead, and though the cause may have been natural or through wounding, in either case we have the parenchyma appearing according to the laws of reversion or retention.

In *Griselinia littoralis* no well-formed parenchyma appeared. However, there are a large number of cells filled with starch which were clearly substitute fibres. These are in a diffuse condition and clearly show that the plant at one time had diffuse parenchyma.

I have shown no other figures of members of the Cornaceae since it is impossible to represent their condition well by a photograph. My specimens of *Mastixia* and of *Helwingia* unfortunately are very small and will not allow any far-reaching conclusions. However, in both instances diffuse parenchyma was found. I hope some time to be able to secure larger specimens, in order to study more thoroughly their structure.

In the stem of *Corokia*, though it shows no well-formed parenchyma, yet the wood cells in certain regions show distinctly the clustered simple pitting so characteristic of the wood parenchyma cells. Often starch also shows in these. They are clearly substitute fibres showing the persistence of the parenchymatous characteristics.

My material of *Aucuba japonica* unfortunately shows no case of wood parenchyma, whether or not it is because the stem and roots were small I cannot be sure. However, the rays are so abundant and so close together that there is scarcely room for, and no use for, parenchyma. Septate tracheides seem to be present to some extent, and many wood cells show nuclei. Here, also, spiral thickenings often appear upon the walls of the tracheides, a condition not at all common to the other members of the family.

Turning now to the Araliaceae, I have been able to study genera including *Aralia*, *Acanthopanax*, *Schefflera*, *Panax*, and *Hedera*. In all cases where parenchyma appears it is clearly vasicentric. Pl. IV, Fig. 10 shows a transverse view of a species of *Schefflera*. It is hard to demonstrate parenchyma from this view. Yet if one looks in the vicinity of the vessels it will be seen that the cells here have thinner and darker walls than those of the general tracheary tissue. The two large vessels just below the centre of the figure show themselves to be nearly enclosed by small cells. Fig. 11 shows a radial section of the same species. Here at each edge a vessel may be seen, and clustered over and about it are rows of parenchyma cells. In Fig. 12, which is also a view of the same species, but is tangential instead of radial, the parenchyma may be seen round the vessels. Here in the centre may be seen a number of septate tracheides. These may be easily told

from the wood parenchyma, since they have very thin cross partitions which lack the middle lamella. In Pl. V, Fig. 13 one may see the radial view of *Acanthopanax senticosus*. Upon the right appears a single row of parenchyma cells adjacent to a vessel. It may be seen that the end wall of the vessel is pierced by a simple elliptical pore. The above characteristics show throughout the genera studied, though in many cases the presence of septate tracheides serves partially to obscure them.

In the last family, the Umbelliferae, I found the same conditions of parenchyma as that characteristic of the Araliaceae. In Pl. V, Fig. 14 we have a transverse view of *Cicuta bulbifera*. At first glance no parenchyma is seen, but upon further study the end walls of the parenchyma cells with their simple pitting may clearly be observed. These cells, it will be noticed, lie adjacent to the vessels. Fig. 15 is that of a tangential view of the same species. Here also the parenchyma shows itself to be only adjacent to the vessels. In Fig. 16, which is a radial view of *Cicuta bulbifera*, we see an example of the vasicentric parenchyma characteristic of the family. It also shows the end pore of the vessel. Here it will be noticed that the pore is rounder, and that the end wall is more at right angles to the lateral walls than was the case with the Araliaceae as shown by *Acanthopanax senticosus*.

In Fig. 17 and in Fig. 18 are seen good examples of diffuse and of vasicentric parenchyma, in cases where such a condition has been recognized. Fig. 17 is that of a transverse view of *Juglans nigra*, and shows the diffuse type, while the vasicentric type is illustrated by the transverse view of *Fraxinus americana* as shown in Fig. 18.

To sum up the results of my study, I find that in Cornaceae the parenchyma, where clearly present in specimens studied, constantly shows a diffuse distribution, and the vessels possess the scalariform type of perforation in their end walls. Native species show parenchyma of varying abundance, but nevertheless quite evident, and the rays small. In exotic genera, on the other hand, the parenchyma, where present, is scarce, and the rays are larger and much more abundant. The wood in the latter case sometimes shows septate tracheides, though they are not at all characteristic of the family. In *Aucuba japonica* spiral thickenings often occur upon the tracheide walls.

In the Araliaceae the parenchyma is always vasicentric. The vessels, moreover, show usually simple elliptical pores in their end walls. These pores are usually at a strongly oblique angle to the lateral walls. Septate tracheides are a common character of the family.

The Umbelliferae show the parenchyma vasicentric as in the case of the Araliaceae. The end wall of the vessels, also, usually show simple pores. Here, however, these are nearly round in circumference, and are nearer at right angles to the lateral walls.

As to the constancy of the scalariform perforations of the vessels in the Cornaceae, I have already noted that simple perforations do occur in a few cases mixed with the scalariform type. In the Araliaceae the vessels have nearly always simple end perforations, though some species also show a few bars. The angle of the perforation is usually quite oblique to the lateral walls, and its circumference is usually elliptical. The Umbelliferae have simple perforations at the ends of the vessels, and these are often round and more at right angles to the lateral walls than in the case of the Araliaceae.

It has already been pointed out that in the sieve-plates of the sieve-tubes, the higher Angiosperms tend to lose their lateral plates, and the end plates come to take up a position more at right angles to the lateral walls (10). This seems to hold true for the perforations of the end walls of the vessels of the higher Angiosperms. I can make no definite statement here without a broader study of the subject, but merely offer this as a suggestion.

#### CONCLUSIONS.

1. That throughout the Cornaceae the parenchyma, where it occurs, is scattered throughout the whole annual ring (diffuse), while throughout the Araliaceae and Umbelliferae it is grouped about the vessels (vasicentric).

2. That the vessels of the Cornaceae show in every species examined, at least in part, scalariform perforations, while all species of the Araliaceae and the Umbelliferae show in part the simple pored condition. Also, that the simple pores of the Araliaceae are more elliptical and more oblique than in the case of the Umbelliferae.

3. That the general anatomical features of the Nyssoideae and Davidioidae do not seem to warrant their being separated from the Cornaceae and their being placed with the Myrtiflorae.

4. That the presence of secretory canals in *Mastixia* is not necessarily of importance in determining the relationship of the genus.

5. Finally that, using the anatomy as a criterion, the Cornaceae should not be placed in the same cohort with the Araliaceae and with the Umbelliferae.

The writer wishes, in closing, to express his gratitude to Professor Fernald, to the Director of the Harvard Botanical Garden, and to the Director of the Arnold Arboretum of Harvard University for specimens. Many of the species studied were secured by Drs. Eames and Sinnott in New Zealand by means of a gift of Mr. J. S. Ames. This investigation has been carried on in the Phanerogamic Laboratories of Harvard University under the direction of Professor Jeffrey, and to him I am greatly indebted for advice and for the photomicrographs accompanying this article.

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DESCRIPTION OF FIGURES IN PLATES IV AND V.

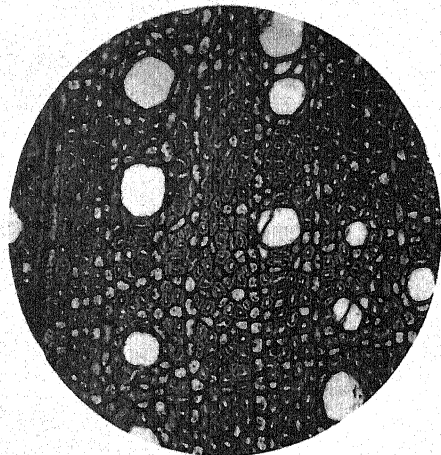
Illustrating Mr. Hoar's paper on Stem Anatomy of the Cohort Umbelliflorae.

PLATE IV.

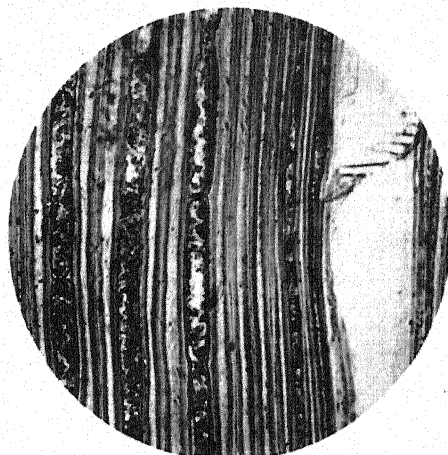
- Fig. 1. *Cornus sanguinea*. Transverse section of stem, showing diffuse parenchyma.  $\times 100$ .
- Fig. 2. *Cornus sanguinea*. Radial section of stem, showing diffuse parenchyma and scalariform vessel.  $\times 250$ .
- Fig. 3. *Nyssa sylvatica*. Transverse section of stem, showing diffuse parenchyma.  $\times 250$ .
- Fig. 4. *Nyssa sylvatica*. Transverse section of stem, showing diffuse parenchyma.  $\times 400$ .
- Fig. 5. *Nyssa sylvatica*. Radial section of stem, showing diffuse parenchyma and scalariform vessels.  $\times 250$ .
- Fig. 6. *Davidia involucrata*. Radial section of stem, showing diffuse parenchyma and scalariform vessels.  $\times 250$ .
- Fig. 7. *Griselinia lucida*. Radial section of stem, showing diffuse parenchyma.  $\times 250$ .
- Fig. 8. *Griselinia lucida*. Transverse section of root, showing tylosis of vessels.  $\times 250$ .
- Fig. 9. *Griselinia lucida*. Radial section of root, showing diffuse parenchyma not noticed in transverse section.  $\times 250$ .
- Fig. 10. *Shefflera* sp. Transverse section of stem, showing vasicentric parenchyma.  $\times 250$ .
- Fig. 11. *Shefflera* sp. Radial section of stem, showing parenchyma clustered over vessels.  $\times 250$ .
- Fig. 12. *Shefflera* sp. Tangential section of stem, showing vasicentric parenchyma and also septate tracheides.  $\times 250$ .

PLATE V.

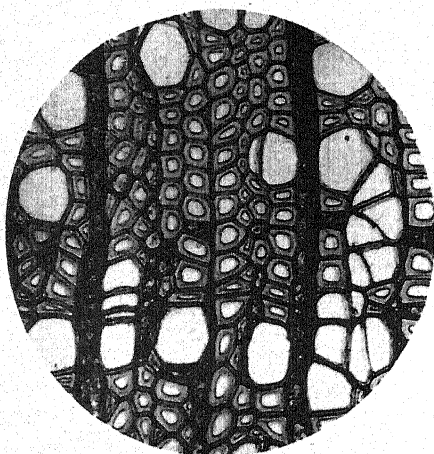
- Fig. 13. *Acanthopanax senticosus*. Tangential section of stem, showing vasicentric parenchyma and vessel with simple elliptical pore placed obliquely to lateral walls.  $\times 250$ .
- Fig. 14. *Cicuta bulbifera*. Transverse section of stem, showing terminal walls of vasicentric parenchyma.  $\times 250$ .
- Fig. 15. *Cicuta bulbifera*. Radial section of stem, showing vasicentric parenchyma and vessel with simple terminal pore.  $\times 250$ .
- Fig. 16. *Cicuta bulbifera*. Tangential section of stem, showing vasicentric parenchyma and vessel with simple round pore in end wall.  $\times 250$ .
- Fig. 17. *Juglans nigra*. Transverse section of stem, showing example of diffuse parenchyma.  $\times 250$ .
- Fig. 18. *Fraxinus americana*. Transverse section of stem, showing example of vasicentric parenchyma.  $\times 250$ .



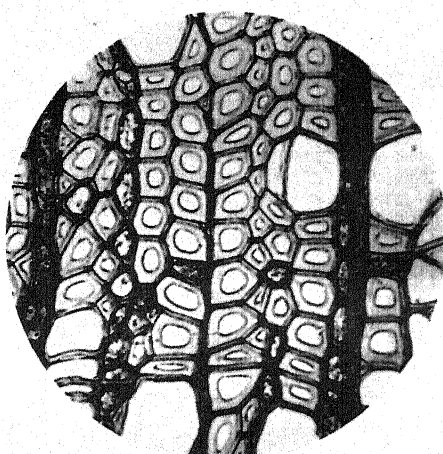
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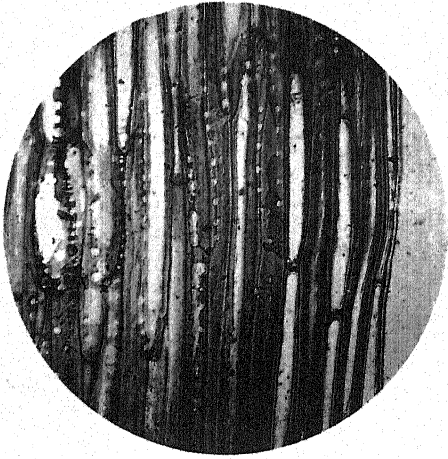
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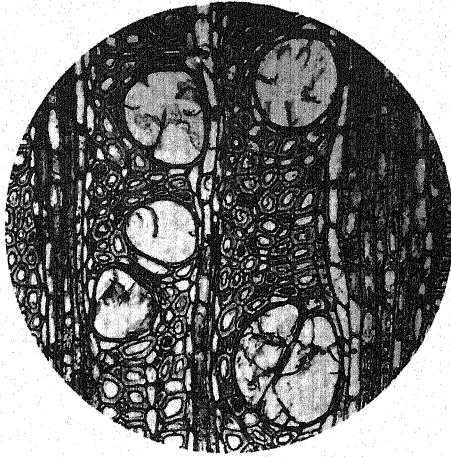
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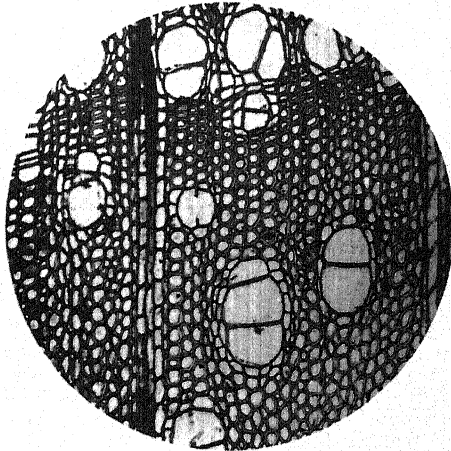
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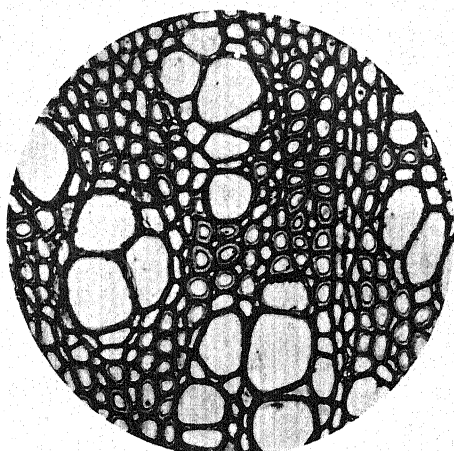
Huth, coll.



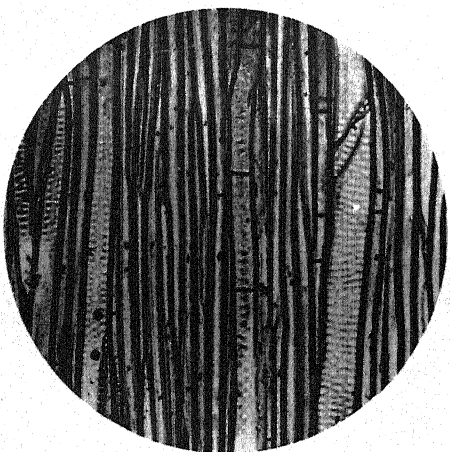




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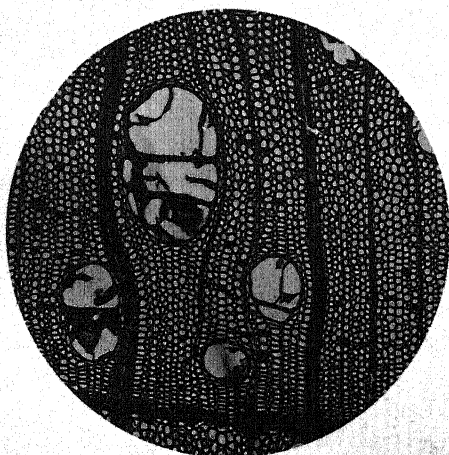
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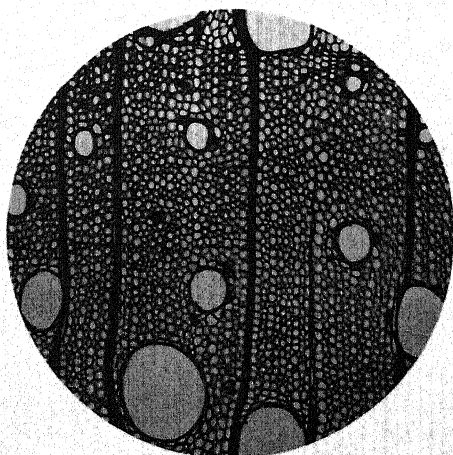
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HOAR — UMBELLIFLORAE.

Huth, coll.



# The Acidity of Sphagnum and its Relation to Chalk and Mineral Salts.

BY

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IN the study of the influence of the chemical nature of the soil on vegetation the question of the effect of calcium carbonate—chalk—has always occupied a prominent position. It is one of the soil constituents which can be most easily recognized and estimated; and the differences in the flora, of which its presence or absence is the cause, are frequently very striking. Lists have been compiled of plants which thrive only on chalk (calcicole), and of others which cannot live in its presence (calcifuge). Such plants may be wholly confined to the one type of soil or to the other; or their behaviour may change with change in other external factors. Reference may be made to Nägeli's ('65) classical case of the calcicole *Achillea atrata*, and the calcifuge *A. moschata*. When the two occur together in the same valley, each is strictly confined to its own type of soil; but if either occurs in absence of the other, it is non-discriminating. Another case of great interest is that of *Castanea vesca*, the Sweet Chestnut, which cannot grow on chalk unless an abnormally high percentage of potassium be present in, or be added to the soil (Arnold Engler, '01).

Perhaps the majority of plants written down as calcifuge belong to this indeterminate type; but there is a number of cases in which the repugnance to chalk is constant, and independent of other external factors. Of these one of the most striking examples is afforded by the genus *Sphagnum*, the members of which are rapidly killed off by water containing calcium carbonate.

Observation of the fact that chalk can exercise so marked an effect on vegetation has led to the attempt to find out exactly in what manner the chalk acts. Such investigation has shown that it acts in a number of quite distinct ways.

Its effect may be indirect. This seems to apply to *Calluna*, studied by M. C. Rayner ('13). She has shown that the presence of chalk interferes with the proper development of the mycorrhiza, and promotes the growth of a bacterial sheath on the roots. This interference with the root symbiosis

is accompanied by symptoms of weakness on the part of the *Calluna*, and is apparently responsible for these.

A more direct influence must be assumed in other cases. This may be either physical or chemical.

Kraus ('11) has demonstrated very completely the effect of chalk on the water content and temperature of the soil; the former is diminished, the latter increased as the amount of chalk present rises. And he has shown that a number of plants typically found on chalk can grow equally well on siliceous soil, if its physical properties resemble those of the chalk.

I am inclined to believe that a case illustrating this is afforded by the distribution of *Helianthemum Chamaecistus* in this country. The Rock Rose is generally described as a calcicole plant (see 'Types of British Vegetation', Tansley, p. 176). It is common, however, and grows well on siliceous gravels in exposed situations in the east of Scotland. In the south of England, where the choice may lie between dry warm chalk and cold wet clay, the former is chosen; where there exists a siliceous soil, which is also well drained and warm, *Helianthemum* can thrive thereon.

Cases in which the chalk acts chemically are also known. The fact that *Castanea* can grow on chalky soil when supplied with an abnormally large amount of potassium, indicates that the chalk acts by interfering with the supply of other salts through the roots. Moreover, the Chestnut grafted on the Oak can grow even on normal chalk soils—a further support of this view (see Jost, '13, p. 125). Schimper ('98) states that in some cases the failure on chalk is due to difficulty in absorbing sufficient iron, and may be obviated by watering with iron solutions.

Another extremely important effect of the chalk is that it alters the reaction of the soil, rendering it neutral or alkaline. To this is probably due its noxious effect in many cases. It will be shown that there are grounds for ascribing its fatal action on *Sphagnum* to this cause.

The earlier observations on *Sphagnum* and its relation to chalk have been collected by Paul ('08), and as most of them have been published in periodicals not readily accessible to English readers, a short summary may be of use.

Sprengel ('47) asserted that *Sphagnum* could not support high concentrations of any mineral substances. Sendtner ('54) was unable to grow it in chalk water, and concluded that basic substances were harmful. Milde ('61) concluded from observations in the field that *Sphagnum* is calcifuge. Pfeffer ('71) notes that it dies in presence of chalk. Öhlmann ('98) found that it dies in a 0.05 per cent. solution of chalk, the time elapsing before death varying with the species used. To produce the same effect with calcium sulphate a solution of twice that strength was necessary, while of calcium nitrate a 0.75 per cent. solution was required. Culture solutions

containing chalk, and tap water (at Bâle, with 0.025 per cent. chalk), were also harmful.

Weber ('00) asserts that he grew *Sphagnum cymbifolium*, *S. fuscum*, *S. acutifolium*, *S. recurvum*, *S. fimbriatum*, *S. platyphyllum* in water containing chalk with success; he even added powdered chalk to his cultures without harming them; *S. recurvum* fruited; only *S. medium* died with powdered chalk, though it lived in chalk water. He concludes that the chalk is harmful only in the presence of other plants, which, growing more vigorously in the chalky water, rapidly supplant the *Sphagnum*.

Graebner ('98, '01, '04) agrees that chalk as such is not harmful, and believes that the failure of the *Sphagnum* is due to too high a mineral content in general. According to Ramann ('95), *Sphagnum* can persist only in water containing less than 0.003-0.004 per cent. of mineral substances.

Düggeli ('03), as a result of experiments carried out on the moor, came to the conclusion that *Sphagnum* was affected adversely not only by chalk, but also by mineral salts in general. His results are not very convincing, as his mineral solutions apparently always contained chalk or magnesia, in addition to other constituents.

Haglund ('12) carried on experiments on the moor at Granarp on a large scale. Table I summarizes his results.

TABLE I.

| Kilos. per Hectare of :           | <i>Sphagnum medium</i> .   | <i>S. rubellum</i> . | <i>S. laxifolium</i> . | <i>S. fuscum</i> . |
|-----------------------------------|--|----------------------|------------------------|--------------------|
| Lime, 6,000 . . . . .             | died   | died                 | died                   |                    |
| Thomas phosphate, 1,000 . . . . . | severely injured   |                      |                        | severely injured   |
| Superphosphate, 400 . . . . .     | died   | died                 |                        |                    |
| Kainit, 1,000 . . . . .           | } induce growth of Mosses and Algae, which cover the young shoots. |                      |                        |                    |
| Potassium nitrate, 400 . . . . .  |  |                      |                        |                    |

By far the most extensive investigation is that carried out by Paul himself ('06, '08). He tested many different salts and many different species of *Sphagnum*, and obtained important results.

In the first place he determined the concentrations of calcium carbonate necessary to kill various species of *Sphagnum*, and arrived at the results given in Table II.

TABLE II.

| <i>Sphagnum</i>     | CaCO <sub>3</sub> in mg.<br>per litre necessary<br>to cause death. | Station.          |
|---------------------|--|-------------------|
| <i>rubellum</i>     | 77   | High moor         |
| <i>papillosum</i>   | 89   |                   |
| <i>molluscum</i>    | 89   |                   |
| <i>medium</i>       | 134  |                   |
| <i>Dusenii</i>      | 171  | High moor ditches |
| <i>acutifolium</i>  | 223  | Wood              |
| <i>platyphyllum</i> | 223  | Low moor          |
| <i>recurvum</i>     | 312  | General           |

The different species are resistant to very different degrees; the dwellers on the high-moor, where the supply of salts is normally very low, are much less resistant than those on the low moor where minerals are more abundant.

Paul then extended his observations to the effects of various salts. All the species enumerated were tested with calcium sulphate; an almost concentrated solution was employed—2 grm. per litre—and all the species grew satisfactorily in it. The other salts were tested with *Sphagnum medium*, a species moderately sensitive to chalk. The concentrations required to kill the Moss were as follows:

Calcium nitrate, 966 mg. per litre.

Potassium bicarbonate, 240; potassium carbonate, 149; sodium bicarbonate, 170; sodium carbonate, 107.

Potassium bisulphate, 720; potassium sulphate, 6,480; sodium bisulphate, 340; sodium sulphate, 5,725; magnesium sulphate, 2,500.

Sodium chloride, about 300; potassium chloride, about 375; calcium chloride, 1,100.

Dipotassium phosphate, 46; monopotassium phosphate, 36; tripotassium phosphate, 34.5.

Sulphuric acid, 150; nitric acid, 82.

Sodium hydroxide, 40.

From this it is evident that we cannot regard *Sphagnum* as being uniformly adversely affected by the high concentrations of the mineral salts applied to it. Some salts it tolerates at high, others are harmful at very low concentrations. The salts of calcium are harmless, while phosphates and alkaline salts appear to be very toxic. It must further be pointed out that, as the salts were tried alone, the important antitoxic action of one salt on another which constantly takes place in nature is omitted; this would probably raise considerably the concentration at which harm would result in a culture solution.

Paul then goes on to discuss the relation of the harmful action of chalk to the 'acidity' of the *Sphagnum*, and here lies the greatest interest of his work. Before discussing it, however, it will be necessary to refer to the investigations of his colleagues Baumann and Gully, and of others, on the nature of the 'acidity' of *Sphagnum* and peat.

In 1906 Count Leiningen ('07) observed that litmus paper applied to *Sphagnum* turned red, and following up this, that *Sphagnum* plants require a considerable quantity of alkali for their neutralization—10 stems 5 cm. long require from 1.3 to 2 c.c. of N/10 NaOH. The degree of acidity cannot be determined by washing out the *Sphagnum* and titrating the wash liquid, as the acid appears to be almost insoluble; it may best be determined by shaking with excess of standard alkali and titrating back with acid.

Baumann and Gully ('10) connected this acidity of *Sphagnum* with the well-known acidity of peaty soils. Peat consists largely of imperfectly decomposed *Sphagnum*, and it seemed probable that the acid of the peat was the acid of the Moss. They determined the acidity of the *Sphagnum* and of the underlying peat, and found in one case :

|                           |       |                                   |
|---------------------------|-------|-----------------------------------|
| <i>Sphagnum</i> . . . . . | 0.228 | gram. acid hydrogen per 100 gram. |
| Peat . . . . .            | 0.260 | gram. acid hydrogen per 100 gram. |

This relation was found to be quite general, the two agreeing closely, with the peat somewhat higher. That the two would be exactly the same was not to be anticipated; the peat contains much foreign matter, and on the other hand the plant remains in it are much altered and partly decomposed.

Baumann and Gully proceeded to investigate the nature of the acid substances. They started with an old observation of Sprengel's, confirmed by various other workers, that peat is able to decompose and render soluble tricalcium phosphate. They worked out the reaction between that compound and peat and *Sphagnum*. Both decompose it in precisely the same way, and to about the same extent. The reaction is very interesting, as the calcium compound is almost insoluble—according to Rindell ('99) 132 mg.  $P_2O_5$  per litre—and it must be supposed that successive small quantities go into solution and are attacked by the organic compounds of the peat (or *Sphagnum*). Part of the phosphoric acid appears in the solution as such, part as monocalcium phosphate; part of the calcium is removed by the peat. The reaction consists essentially of a splitting up of the phosphate with removal of the base and liberation of the acid. The formation of the monocalcium salt may be regarded as secondary, due to action between the liberated acid and the undecomposed phosphate.

To show the extent of the solvent action we may give the following figures :

|                                     |       |                |                         |                |                     |
|-------------------------------------|-------|----------------|-------------------------|----------------|---------------------|
| Of tricalcium phosphate with        | 1,200 | c.c. water,    | 158                     | mg. $P_2O_5$ . | } go into solution. |
| "                    "            " | 1,200 | c.c. water + 6 | gram. <i>Sphagnum</i> , | 1,383          | mg. $P_2O_5$ .      |
| "                    "            " | 1,200 | c.c. water + 6 | gram. Peat,             | 1,561          | mg. $P_2O_5$ .      |

The observations were extended to other salts, and the remarkable result was obtained that, with the exception of the extremely insoluble calcium oxalate, all the salts tried were broken up with liberation of the acid of the salt. The amount of acid hydrogen liberated from the various salts tested, by 100 gram. dry peat or *Sphagnum*, is given in Table III.



TABLE III.

| Salt.                | <i>Sphagnum.</i> | Peat.               |
|----------------------|------------------|---------------------|
| Sodium chloride . .  | 0.0122           | 0.0199              |
| Potassium sulphate . | 0.0207           | 0.0253              |
| Calcium chloride .   | 0.0144           | 0.0162              |
| Ammonium sulphate    | 0.0253           | 0.0300              |
| Potassium iodide .   | 0.0119           | 0.0120 <sup>1</sup> |
| Sodium nitrate . .   | 0.0224           | 0.0205              |
| Sodium sulphite .    | 0.0778           | 0.1092              |
| Sodium formate .     | 0.0706           | 0.0852              |
| Sodium butyrate .    | 0.0675           | 0.0836              |
| Sodium salicylate .  | 0.0491           | 0.0563              |
| Ammonium acetate     | not determined   |                     |
| Calcium acetate .    | 0.0840           | 0.01090             |

It will be seen that the two substances possess the property in common, and that, while the peat is slightly more active, the two sets of figures run so closely parallel that it would seem permissible to refer the property to the possession of some common compound.

Baumann and Gully fix on the fact that the *Sphagnum* and peat are able to break up such salts as sodium chloride, liberating the acid and removing the base. They say that if an acid is responsible, then we must suppose that an insoluble organic acid is capable of breaking up so strong a combination as sodium chloride, producing an insoluble sodium salt, and liberating hydrochloric acid. This they consider impossible. They believe that the reaction is due to the presence of *colloidal* substances which *adsorb* the base and set free the acid. They adduce the following considerations in support of their theory:

1. The conductivity of *Sphagnum*<sup>1</sup> is very low, only about 1/10 of that of a solution of acetic acid having the same solvent action on tricalcium phosphate.

2. When *Sphagnum* acts on a salt the amount of acid liberated is, relatively to the amount of *Sphagnum* employed:

(a) Less as the concentration of the solution is decreased ;<sup>2</sup>

(b) Greater as the amount of *Sphagnum* acted on by a constant volume of solution, is decreased.

In the case of the combination of an acid and base giving an insoluble salt, the amount of salt formed would be directly related to the amount of the reagent present in smaller quantity, in this case of the *Sphagnum*.

3. The activity of *Sphagnum* decreases slowly when it is kept ; this would correspond to the slow change in surface of a colloid.

<sup>1</sup> The alternative 'peat' is implied.

<sup>2</sup> For tricalcium phosphate aberrant results were obtained : these do not agree with the results of Tacke and Süchting, or of Fleischer (Landw. Jahrb. 1883, vol. xii, p. 164) ; it would seem that none of these investigators has paid sufficient attention to the complicated nature of this particular reaction, and that slight differences in method may be responsible for the discrepancies. But for chlorides a maximum absorption was found in normal solutions.



4. Reference to Table III shows that the amounts of the acids set free from different salts are widely different. It is found that, for a series of salts with the same base, acids are liberated in the following order: (a) hydrobromic, hydriodic, hydrochloric, nitric; (b) sulphuric; (c) acetic, the last named in largest quantity. This corresponds to the activity of the acids in various colloidal reactions.

5. As regards the bases, bivalent bases are adsorbed more actively than monovalent, potassium more than sodium.

6. It is possible to wash out a large portion of the adsorbed base with distilled water, especially if it contain carbon dioxide. By this means the original acidity of the *Sphagnum* may be almost completely restored.

Baumann and Gully conclude that the old 'humus acids',<sup>1</sup> to which the acidity of acid soils in general, and of peat in particular, was ascribed, are non-existent: the acidity is in reality due indirectly to the presence of negatively charged colloidal compounds; these break up any salts present in the soil, and the acid of the salt produces an acid reaction in the soil. They regard the colloids in question as being chiefly located in the hyaline cells of the *Sphagnum* leaf.

Since the publication of Baumann and Gully's paper a number of others have appeared supporting or criticizing their conclusions.

Czapek ('11) agrees with the authors in all their deductions. Wieler ('12) also supports the colloid hypothesis.

On the other hand, a number of chemists have attacked these views, and sought to refer the reactions to ordinary chemical processes with typical acids. The most important papers are those of Tacke and Süchting ('11), Tacke, Densch, and Arndt ('13), Rindell ('11), Odén ('12), Ehrenberg and Bahr ('13). Gully ('12) has replied to some of those criticisms. The matter is really one for the physical chemist and it is impossible to go into details, but a few of the more important points may be summarized.

Tacke and Süchting dispute some of Baumann and Gully's experimental data; refer the phenomena with tricalcium phosphate to interaction between humus acids, phosphoric acid, and the phosphates; find that drying to 130°C., and consequent serious diminution of the colloidal adsorptive surface, has no influence on the amount of acid liberated; that peat can invert cane sugar, and give off hydrogen with iron—two typical acid reactions; and they can find no parallel to the reactions using other typical colloids, such as starch and gelatine. Colloid action is to be observed only in the adsorption of colloidal ferric hydroxide; all the other reactions are to be referred to the action of humus acids as such.

<sup>1</sup> The form 'humus' is preferred to 'humous' or 'humic', as the terminations of these are associated with definite chemical constitutions.

Rindell criticizes from the physico-chemical standpoint, and finds the reactions explicable on the assumption of a mixture of more and less soluble humus acids.

Odén and Ehrenberg and Bahr point out that it is scarcely permissible to apply fine methods, such as that of conductivity determination, to so coarse a mixture as that presented by ordinary peat. They attempt to isolate the humus acids by extraction with ammonia, precipitation with acid, and further purification.

Conductivity determinations with a preparation thus obtained led Odén to the conclusion that its combination with ammonia is of the nature of a true salt formation. He roughly determined its equivalent weight and basicity.

Ehrenberg and Bahr, with an improved preparation, confirmed these results. They also attempt to demonstrate the true chemical nature of the compound with ammonia, by observations on its thermic decomposition, and by comparing the adsorption of ammonia with that of sulphur dioxide. Their experimental results do not, however, seem capable of an interpretation on the assumption that only a simple chemical reaction is involved. They suppose that the compounds formed with bases go into solid solutions with uncombined humus acids, and so account for aberrant numerical results. This, however, seems to be an approach to the views held by Baumann and Gully. Very important is the fact that their insoluble preparation of humus acids is capable of decomposing tricalcium phosphate, so that it possesses one at least of the peculiar properties of the natural compounds.

It is clear that in the view of chemists the theory of Baumann and Gully is by no means held to be proved; but at the same time evidence as to the existence of *insoluble* alkaline salts of the humus acids is not forthcoming. The compounds of the artificial preparations with the alkalis are soluble; in fact on this depend the various methods for their preparation. To account for the retention of the bases in the form of such salts by the peat or *Sphagnum*, some sort of *adsorption* must be called into play. Ehrenberg and Bahr admit as much when they invoke the aid of 'solid' solutions to explain their figures; and, as a matter of fact, no one denies that the humus acids are colloids. Acids which are colloids will act both as acids and as colloids. The attempt to explain all their peculiarities on the basis of one only of these two properties is bound to lead to failure.

In what follows the terms 'acid' and 'humus acid' are employed only because they denote most conveniently the chief property of the substances in question—their responsibility, direct or indirect, for an acid reaction; this use does not imply agreement with the view that they do not act also as colloids.

For the purposes of the ecologist it is sufficient to recognize that peat contains compounds capable of breaking up salts and liberating their acids,

and that the acid nature of peaty soil is probably largely due to the presence in it of these (mineral) acids. These compounds are already present in *Sphagnum*. The significance of these for the life of the *Sphagnum* is supposed by the authors to lie in the possibility it gives of absorbing bases from the very dilute solution in which the Bog-moss lives. They further suggest that the property is not confined to *Sphagnum*, but that the absorption of mineral salts by the root-hairs of the higher plants may take place in the same way.

To return to Paul's researches on the relation of *Sphagnum* to chalk. He suggests that the chalk saturates the acid compounds of the cell-walls, and so prevents the absorption of bases. This does not result directly in death from starvation, but it causes the plant to make an effort to replace the saturated compounds, with the result that metabolism is so much increased that death results from a sort of exhaustion.

In support of this he brings forward the fact that different *Sphagna* have different acidities, and that hand in hand with this variation goes the variation in repugnance to chalk; the more acid species being also the more sensitive. Table IV gives the acidity of the various species in grams of acid hydrogen per 100 grm. *Sphagnum*, as determined by titration with N/4 NaOH. And along with these is given the quantity of calcium carbonate required to kill 1 grm. (dry wt.) of each species.

TABLE IV.

| <i>Sphagnum</i>           | Acidity. | Chalk fatal<br>(in mg.). |
|---------------------------|----------|--------------------------|
| <i>rubellum</i> . . .     | 0.120    | 62.55                    |
| <i>medium</i> . . .       | 0.104    | 59.93                    |
| <i>teres</i> . . .        | 0.102    | 172.00                   |
| <i>papillosum</i> . . .   | 0.101    | 60.02                    |
| <i>molluscum</i> . . .    | 0.098    | 69.51                    |
| <i>fuscum</i> . . .       | 0.096    | 68.80                    |
| <i>cuspidatum</i> . . .   | 0.093    | 75.18                    |
| <i>acutifolium</i> (moor) | 0.090    | 78.33                    |
| <i>cymbifolium</i> . .    | 0.086    | 125.15                   |
| <i>acutifolium</i> (wood) | 0.083    | 92.71                    |
| <i>contortum</i> . . .    | 0.081    | 155.25                   |
| <i>Girgensohnii</i> . .   | 0.079    | 121.33                   |
| <i>recurvum</i> . . .     | 0.076    | 126.48                   |
| <i>parvifolium</i> . .    | 0.074    | 185.47                   |
| <i>platyphyllum</i> . .   | 0.060    | 321.98                   |

The agreement between high acidity and great sensitiveness is very close; only *Sphagnum teres*, with the high acidity of 0.102, has also a great power of resistance to chalk, coming in this respect third from the end of the list. The amounts of chalk are given, not in terms of the concentration employed, but as the number of mg. required to kill 1 grm. of the plant (dry wt.). If concentration is taken, the agreement, although it still holds in a general way, is not so satisfactory, as may be seen by a

comparison with Table II. Paul prefers the former method of statement, but does not show experimentally that the toxic effect of the chalk takes place when a certain amount is supplied, rather than when a certain concentration of the solution is reached.

On the hypothesis that the presence of the humus acids is largely responsible for the supply of mineral nutrients, Paul argues that the *Sphagna*, which inhabit stations where the supply of salts is very low, will contain the largest quantities of these compounds. This is so: *S. rubellum*, the typical high moor *Sphagnum*, stands first, and the degree of acidity falls away in species which inhabit more favoured stations. The effect of the chalk is to neutralize the acids and render them incapable of absorbing further mineral supplies. In the high moor species, which are most dependent on their acids, this interferes more intensely with the normal metabolism of the plant, and these species are consequently the more sensitive.

The experiments to be described were commenced on the publication of Baumann and Gully's memoir, with the intention of trying over some of their results, and were subsequently extended to include some aspects of the work of Paul.

#### I. LIBERATION OF ACIDS FROM THEIR SALTS.

This fundamental effect is very readily demonstrated. It is only necessary to soak a few shoots of *Sphagnum rubellum* for a few hours in a solution of any salt—say 5 per cent. NaCl—and then to test with methyl orange: a strong acid reaction is always obtained. A control with distilled water always gives a negative result with methyl orange, though a slight reaction may be obtained with litmus; as this is reversed on boiling, it may be taken as due to the presence of carbon dioxide.

That the acid present in the treated salt solution is the acid of the salt employed is not so easily demonstrated. The following method is fairly conclusive. Solutions of copper chloride of 0.5 %, 0.1 %, 0.05 %, 0.025 %, are employed; of each 100 c.c. is allowed to stand overnight with about 5 grm. (moist) of *Sphagnum*. Each solution is then tested with ammonium hydroxide, and the colour produced compared with that given by control portions of the original solutions. It is found that the colour given by the stronger solutions is much weakened, by the two weaker almost if not quite gone. If the chloride be tested for with silver nitrate, then the amount of the precipitate is found to be the same before and after treatment. A large amount of copper has thus been removed, while the acid radicle is present in undiminished quantity and the solution has acquired a strong acid reaction. The presence of copper in the *Sphagnum* may be demon-

strated by washing free from the cuprous solution and treating with ammonia; the leaves, and especially the thicker stems, take on a marked blue-green colour.

## II. LOCALIZATION OF THE ACID COMPOUNDS.

Baumann and Gully express the opinion that the walls of the hyaline cells are the chief seat of the colloids, but they do not give any experimental evidence in support of this view. The attempt was made to find out whether the compounds were located in any particular position in the plant.

The leaves were carefully teased off a number of *Sphagnum* plants, and the leaves and leafless stems placed separately in 5 per cent. NaCl solution. Both gave a strong acid reaction.

Quantitative determinations show that the stem is very slightly more active than the rest of the plant. As the method employed was the same in all quantitative determinations it may be described here. It was found impossible to treat directly with sodium hydroxide, as the resulting solution was frequently too dark to admit of accurate titration. Consequently the method of estimating the acid liberated from a salt was employed. 10 per cent. calcium acetate is the most advantageous salt; according to Baumann and Gully the amount of acetic acid set free is equal to 95 per cent. of the acidity of the *Sphagnum*, as indicated by direct treatment with sodium hydroxide. The solution obtained is almost colourless, and may be accurately titrated with phenolphthalein and hydroxide. 1.5 gram. of dry *Sphagnum* was shaken for six hours with 100 c.c. of the solution, and the acid determined in the liquid strained off through muslin. The acid is expressed in grammes of acid hydrogen per 100 gram. of dry *Sphagnum*. The figures are thus comparable with those of the former investigators which are expressed in the same way.

Two lots of *Sphagnum cymbifolium* were treated in this way; the one (a) consisted of small branches and leaves, the other (b) of carefully selected stems. The acidities were:

(a) 0.080,

(b) 0.085.

The difference is small, but it was obtained in four further experiments. The slightly greater acidity of the stems might be referred to the greater thickness of the cell-walls.

That the acid reaction is not connected with the life of the plant scarcely needs proof; it is given by plants in the fresh state, and by plants dried for several hours at temperatures of over 100° C. From this, however, it does not follow that the reaction is due to the wall rather than to the cell contents.

A method described by Czapek ('99) for getting rid of cell contents was tried. It consists in boiling under the reversed condenser first in ether, then in alcohol, and finally in distilled water. Such substances as fats and chlorophyll are thus removed. The treatment has no effect on the acid nature of the plant. *S. acutifolium* gave a strong reaction with sodium chloride after the treatment.

The cell contents, including the protoplasm, may be almost entirely removed by chloral hydrate (5 parts to 2 aq. dist.). *S. rubellum* was digested with chloral hydrate for ten days, thoroughly washed out, and then tested for acid. The results were :

Untreated, 0.0947,

Treated, 0.0625.

Examination under the microscope showed only shrivelled remains of the cell contents. The acidity is reduced to two-thirds, and this at least must be due to substances in the cell-wall. It is probable that the third, which has disappeared, is not due to cell contents, but to cell-wall also; for this must undergo some alteration under the influence of the powerful reagent.

### III. OCCURRENCE OF THE ACID REACTION IN OTHER PLANTS.

Baumann and Gully state that several other Mosses have the same properties as *Sphagnum*. I tested a number of Mosses with 5 per cent. salt solution and obtained the reaction with *Polytrichum strictum*, *P. commune*, *Leucobryum glaucum*, *Hypnum splendens*, *Hylocomium loreum*; *Fontinalis antipyretica* gave a faint reaction. In addition to these, the Lichens *Parmelia laevigata*, *Evernia furfuracea*, *E. prunastri*, *Usnea barbata*, all gave a strong reaction. No reaction, on the other hand, was given by *Mnium hornum*, *Neckera pennata*, *N. crispa*, *Hylocomium triquetrum*, *Leucodon sciuroides*.

The reason for the negative result may be threefold. (a) The acid compounds of the moss may be saturated already. *Leucodon*, after washing out with CO<sub>2</sub> water, gave a strong reaction, while the reaction of *Fontinalis* was increased. *Weissia viridula*, a chalk Moss, could be freed from adhering particles only by washing with dilute HCl, and then water; thereafter it gave a strong reaction. (b) Those objects which have a low acidity may be unable to liberate sufficient HCl from sodium chloride (an unfavourable salt) to give the reaction. The behaviour of different *Sphagna* illustrates this. Salt solution in which *S. rubellum* has been soaked gives a very strong reaction; if *S. recurvum* has been used the reaction is less marked, while after *S. contortum* it is slight. With *contortum* which has been washed it is stronger. (c) There is, in the third place, the possibility of a specific difference between the acid substances in different plants.

*Sphagnum contortum*, with an acidity of 0.0276, gives a weak but very distinct reaction with salt; while *Fontinalis*, with an acidity of 0.0414, gives only a very faint reaction. This would seem to indicate that while *S. contortum* is more efficacious than *Fontinalis* in breaking up salt, the reverse is the case with calcium acetate.

The qualitative test with sodium chloride is not delicate, and all further tests were made quantitative with calcium acetate. These were not confined to the Mosses. Wieler ('13) states that the properties of *Sphagnum* are exhibited by the cell-walls generally of vascular plants. Confirmation of this was desirable and was obtained. The results are set out in Table V. In the cases of those plants marked x a control experiment was carried out, using distilled water. In every case the water was neutral at the end of the experiment. This disposes of Arndt's suggestion that the acidity in these cases is due to soluble organic acids originally present in the plants.

TABLE V.

| Plant.                           | Parts tested.           | Acidity. |
|----------------------------------|-------------------------|----------|
| <i>Dicranum scoparium</i> .      |                         | 0.0541   |
| <i>Fontinalis antipyretica</i>   |                         | 0.0414   |
| <i>Neckera complanata</i> .      |                         | 0.0367   |
| <i>Mnium hornum</i> . .          |                         | 0.0310   |
| <i>Polytrichum commune</i>       |                         | 0.0299   |
| <i>Evernia prunastri</i> .       |                         | 0.0588   |
| <i>Fagus</i> . . . . .           | dead leaves x           | 0.0345   |
| <i>Pinus</i> . . . . .           | dead needles x          | 0.0230   |
| <i>Pteris</i> . . . . .          | dead fronds x           | 0.0230   |
| <i>Aira</i> . . . . .            | dead stems and leaves x | 0.0195   |
| <i>Calluna</i> . . . . .         | dried shoots x          | 0.0149   |
| Orchid air roots (sp. not known) |                         | 0.0172   |

Wieler states that cellulose as cotton-wool, and as a preparation from wood, is also acid; I tested cotton-wool and filter-paper several times with uniformly negative results. It is scarcely conceivable that they should be able to absorb bases from salts, and could they do so our entire chemical data would stand in need of revision!

The acidity of *Sphagnum* is on the average 0.07, so that most of the other objects tested are less active, and lie in or below the region of the less active species of the Bog-moss.

The acid properties are widely distributed throughout the vegetable kingdom, and to them is certainly to be attributed the acid nature of humus. The action of chalk on the soil, besides the direct neutralization of acids therein, will be to saturate the acid compounds of the plant remains and so to prevent them breaking up salts in the soil solution; that is, it prevents the souring of the soil indirectly as well as directly. It need scarcely be mentioned that the presence of such compounds in the roots may have a most important bearing on the absorption of mineral nutrients.

## IV. THE VARIATION OF ACIDITY IN DIFFERENT SPHAGNA.

Paul pointed out that the different *Sphagna* exhibit different degrees of acidity. It seemed possible that the differences were secondary and not primary. Were all the species of the same initial acidity, then those which live in stations where the mineral supply is low would absorb less base than those in more favourable stations, would retain more unsatisfied acid, in other words they would appear to be more acid.

I tested this theory by washing out the absorbed bases and determining the acid in the washed material. 3 grm. of dry *Sphagnum* was shaken with 1 litre of distilled water saturated with carbon dioxide from a Sparklet bottle; the water was changed thrice, and the washing lasted in all forty-eight hours. By this means about 90 per cent. of the absorbed base may be washed out. The residue was dried, and the acidity determined in the usual way. A considerable number of species was investigated, and material of each was obtained from as many localities as possible. The results are set down in Table VI.

The difference in acidity is primary; the washing out constantly increases the acidity, but almost no general levelling up between the different species takes place. On the whole they retain their relative positions, and probably the order would be still more nearly the same if a larger number of determinations were made, for the individual differences are considerable. This goes to strengthen the conclusion of Paul, that those species which live in stations poor in food-stuffs require the highest acidities in order to obtain the necessary amount of bases. Moreover, the amount of absorbed base, which is equivalent to the amount of the acid saturated, may be obtained by subtracting the secondary from the primary acidity. The results are given in the sixth column of the Table, and it is seen that the values for the various species lie quite close together. In particular the variations are scattered through the series; there is no progressive increase in the amount of saturated acid with increase in acidity; that is to say, the different species, by virtue of their different acidities, are able to hold approximately the same amount of base in reserve.

In Paul's Table the acidity seems, with one exception, to have been determined in a single sample. The differences between the species are small, frequently smaller than the differences between samples of one species as shown in my determinations. The samples of one species agree only moderately well; it is certainly impossible to get a value holding for all samples of a species, and it is scarcely permissible to take the acidity of a single sample as representative of the species as a whole.

Further, Paul's results were obtained by titration with sodium hydroxide. Rindell ('11) has remarked on the impossibility of obtaining an exact end-point with this method; I, too, found that in many cases the solutions



TABLE VI.

| <i>Sphagnum</i>            | <i>Acidity in grammes acid hydrogen per 100 grm. Sphagnum.</i> |                          |                        |        |
|----------------------------|--|--------------------------|------------------------|--------|
|                            | <i>Unwashed (secondary).</i>                                   | <i>Washed (primary).</i> | <i>Saturated Acid.</i> |        |
| <i>rubellum</i>            |  |                          |                        |        |
| Maryculter . . . .         | 0.0885   | 0.1161                   |                        |        |
| Maryculter . . . .         | 0.0896   |                          |                        |        |
| Maryculter . . . .         | 0.0863   | 0.0906                   | 0.1092                 | 0.0186 |
| Countesswells . . . .      | 0.0977   | 0.1125                   |                        |        |
| Ullapool . . . . .         | 0.0947   | 0.1161                   |                        |        |
| Glen Dee . . . . .         | 0.0981   | 0.1034                   |                        |        |
| Aberdeen . . . . .         | 0.0793   | 0.0977                   |                        |        |
| <i>acutifolium alpinum</i> |  |                          |                        |        |
| Cairngorms . . . .         | 0.0653   | 0.1081                   |                        |        |
| Cairngorms . . . .         | 0.0751   | 0.0988                   |                        |        |
| Cairngorms . . . .         | 0.0906   | 0.1057                   | 0.1080                 | 0.0210 |
| Cairngorms . . . .         | 0.1104   | 0.1115                   |                        |        |
| Rothiemurchus . . . .      | 0.1034   | 0.1211                   |                        |        |
| <i>cymbifolium</i>         |  |                          |                        |        |
| Aberdeen . . . . .         | 0.0666   |                          |                        |        |
| Southampton . . . .        | 0.0815   | 0.1039                   |                        |        |
| Skene . . . . .            | 0.0850   | 0.1103                   | 0.0986                 | 0.0225 |
| Countesswells . . . .      | 0.0715   | 0.0816                   |                        |        |
| Maryculter . . . . .       | 0.0747   |                          |                        |        |
| <i>papillosum</i>          |  |                          |                        |        |
| Lochnagar . . . . .        | 0.0717   | 0.0885                   |                        |        |
| Aberdeen . . . . .         | 0.0609   | 0.0866                   |                        |        |
| Ullapool . . . . .         | 0.0740   | 0.0893                   | 0.0881                 | 0.0193 |
| <i>acutifolium</i>         |  |                          |                        |        |
| Scotston . . . . .         | 0.0712   |                          |                        |        |
| Orkney . . . . .           | 0.0545   | 0.0862                   | 0.0862                 | 0.0206 |
| Mossbrodie . . . . .       | 0.0712   |                          |                        |        |
| <i>subsecundum</i>         |  |                          |                        |        |
| Lochnagar . . . . .        | 0.0660   | 0.0830                   |                        |        |
| Glen Dee . . . . .         | 0.0524   | 0.0827                   | 0.0828                 | 0.0236 |
| <i>recurvum</i>            |  |                          |                        |        |
| Maryculter . . . . .       | 0.0517   | 0.0707                   |                        |        |
| Countesswells . . . .      | 0.0717   | 0.0885                   | 0.0792                 | 0.0178 |
| Skene . . . . .            | 0.0609   |                          |                        |        |
| <i>squarrosum</i>          |  |                          |                        |        |
| Countesswells . . . .      | 0.0643   | 0.0620                   | 0.0793                 | 0.0167 |
| Skene . . . . .            | 0.0597   | 0.0781                   |                        |        |
| <i>laricinum</i>           |  |                          |                        |        |
| Southampton . . . .        | 0.0501   | 0.0566                   | 0.0781                 | 0.0215 |
| Scotston . . . . .         | 0.0632   |                          |                        |        |
| <i>cuspidatum</i>          |  |                          |                        |        |
| Aberdeen . . . . .         | 0.0448   |                          |                        |        |
| Rothiemurchus . . . .      | 0.0464   | 0.0666                   |                        |        |
| Fochabers . . . . .        | 0.0487   | 0.0781                   | 0.0312                 |        |
| Southampton . . . .        | 0.0368   | 0.0896                   |                        |        |
| Fochabers . . . . .        | 0.0580   | 0.0787                   |                        |        |
| <i>compactum</i>           |  |                          |                        |        |
| Ullapool . . . . .         | 0.0460   |                          |                        |        |
| Bristol . . . . .          | 0.0563   | 0.0555                   | 0.0745                 | 0.0190 |
| Carlisle . . . . .         | 0.0643   |                          |                        |        |
| Cairngorms . . . . .       | 0.0453   |                          |                        |        |
| Rothiemurchus . . . .      | 0.0563   | 0.0687                   |                        |        |
| Cairngorms . . . . .       | 0.0648   | 0.0804                   |                        |        |
| <i>contortum</i>           |  |                          |                        |        |
| Ullapool . . . . .         | 0.0506   | 0.0816                   |                        |        |
| Countesswells . . . .      | 0.0565   | 0.0712                   |                        |        |
| Countesswells . . . .      | 0.0506   | 0.0678                   | 0.0715                 | 0.0226 |
| Cairngorms . . . . .       | 0.0591   | 0.0793                   |                        |        |
| Skene . . . . .            | 0.0276   | 0.0597                   |                        |        |

were so dark or so ruddy that any approach to accuracy was quite out of the question. With calcium acetate, on the other hand, accurate values may be almost always obtained.

There is reason to believe that the acidity of a single sample varies throughout the year. If the acid compounds really act as absorbers of salts, which are then used up in growth, continued absorption during winter, when growth is at a standstill, will result in a relatively smaller acidity. A sample of *S. recurvum* gave an acidity of 0.0517 in early April. A large tuft was placed in a 2-litre bottle with distilled water, and after six weeks had increased in length by 2-3 cm. The new growth was cut off and the acidity determined in it and in the older parts; the latter gave 0.0678, the former 0.0758. This indicates that during growth minerals are actually removed from the older parts; while, growing in distilled water, the young shoots, not being able to absorb bases, have a very high acidity. But it also shows that in normal conditions the acidity will vary greatly according to salt supply and rate of growth, and that consequently the difficulty of obtaining a value characteristic of a species is increased. Paul's Table (IV) should thus be taken with some caution. The order in which Table VI places the species does not agree well with that of Paul, but it might be materially altered by selecting individual determinations; in any case a comparison is not profitable, as the number of species studied in common is not sufficiently large.

Tacke and Süchting state that the acidity of *Sphagnum* cannot be increased by washing out. I obtained an increase in every case; the discrepancy may be due to the use of ordinary distilled water containing only a small quantity of carbon dioxide by these authors.

#### V. SPHAGNUM AND CHALK.

The relation to calcium carbonate may be discussed under two different heads: (a) Is the sensitiveness different in the different species? (b) In what does the toxic action of chalk consist?

(a) If we place *S. rubellum* and *S. contortum* in water containing 100 mg. of calcium carbonate to the litre, we find that in a day or two the former has turned a dirty blackish colour, and that after a fortnight or three weeks it has died and fallen to pieces without having grown in the least; *contortum*, on the other hand, remains bright green, exhibits geotropic movements, and adds considerably to its length. This illustrates the fact that different species are sensitive in different degrees. The relation of growth to calcium carbonate was investigated more exactly in the case of three species of widely different acidity:

|                       |        |                  |
|-----------------------|--------|------------------|
| <i>S. contortum</i> , | 0.0276 | 0.0597 (primary) |
| <i>S. recurvum</i> ,  | 0.0517 | 0.0707 (primary) |
| <i>S. rubellum</i> ,  | 0.0863 |                  |

The sample of *contortum* used had a remarkably low acidity, only about half that usually shown by this species.

The plants were grown in 600 c.c. conical Jena glass flasks, in 300 c.c. of solution. Ten plants 5 cm. long were grown in each flask, and every culture was duplicated, so that the results are averages for twenty plants. The cultures lasted five weeks, from the middle of March to the end of April, and were kept in an unheated room. All cultures subsequently described were carried out in precisely the same way. The results are calculated in percentages of the growth in distilled water; what that is for the various species may be seen from the following figures:

*S. contortum*, 4.6 cm.  
*S. recurvum*, 2.25 cm.  
*S. rubellum*, 0.75 cm.

The small growth of *rubellum* makes it impossible to regard small differences in its case as significant.

The results of the cultures in chalk solutions are given in Table VII.

TABLE VII.

| CaCO <sub>3</sub> in mg.<br>per litre. | Growth of <i>Sphagnum</i> |                   |                   |
|--|---------------------------|-------------------|-------------------|
|  | <i>contortum</i> .        | <i>recurvum</i> . | <i>rubellum</i> . |
|  | %                         | %                 | %                 |
| 50                                     | 84                        | 47                | 0                 |
| 75                                     | 57                        | 49                | 0                 |
| 100                                    | 44                        | 51                | 0                 |
| 125                                    | 30                        | 24                | 0                 |
| 150                                    | 11                        | 16                | 0                 |
| 175                                    | 4                         | 14                | 0                 |
| 200                                    | 0                         | 11                | 0                 |
| 225                                    | 0                         | 9                 | 0                 |
| 250                                    | 0                         | 9                 | 0                 |

From this it appears that *contortum* is the least sensitive, though *recurvum* shows a very slight growth in high concentrations. The geotropic reaction persists in *contortum* up to 150 mg., while in *recurvum* it disappears at 100; the latter is also much more severely bleached: *rubellum* is the most severely affected.

The investigations of Paul on this point have been described in detail.

The experiment quoted in Table VII shows: (1) that the species studied are sensitive to chalk in different degrees; (2) that the more acid species are the more sensitive. Paul's thesis is thus confirmed in principle. But the very exact parallel which he finds must be criticized on several grounds. In practice it is not possible to determine either acidity or sensitiveness with the degree of accuracy which Paul suggests.

As far as can be gathered from his paper, it would seem that the samples used for acidity determinations were the same as those used for

chalk cultures. So that, though his acidity values are not characteristic for the species, this does not invalidate, but rather enhances the value of his conclusions as to the connexion between acidity and sensitiveness. The lack of accuracy of his method of determining acidity is, however, a serious objection.

When we turn to the determination of the fatal dose of chalk, we find that it is a matter of great difficulty. This is at once evident on reading Paul's description of the behaviour of almost any species in different concentrations of chalk solution. The damage increases with concentration, but quite gradually; this applies to the decrease in the amount of growth, the discoloration, and even to the inhibition of the geotropic reaction. The effect on growth is exemplified in Table VII, and the other symptoms may be well seen in such cultures of *recurvum*. Even in the highest concentrations the tips may remain fresh, green, and alive. Besides the species mentioned, *papillosum*, *subsecundum*, *squarrosus*, and *cymbifolium* were examined, but only in the case of *rubellum* could anything like a sharp limit be obtained. The conclusion is inevitable that the designation of any particular concentration as initiating fatal damage must be largely arbitrary.

As already mentioned, Paul states his chalk as *amount* per 100 grm. dry weight of *Sphagnum*, instead of as *concentration* of the solution employed. To test the validity of the assumption that it is the actual amount supplied, and not the concentration of the solution employed, that is determinative, two sets of *recurvum* were grown, one in 2,000 c.c., the other in 200; the chalk present in each was the same—200 mg., so that only the concentration was different. The first set showed a growth of 37 per cent., a strong geotropic curvature, and a fairly healthy colour; the second did not grow at all, showed no curvature, and was quite dead. From this it follows that it is the high concentration that is effective.

Paul shows that even in the case of *rubellum* the amount of chalk supplied must be sufficient to neutralize the acid compounds before damage sets in. Were such small quantities of solution employed that the amount of chalk therein was not sufficient to neutralize the *Sphagnum* employed, then *amount* would enter as a factor. When, as in my experiments, an excess is present even in the dilute solutions, then only *concentration* will come into play. Paul's paper gives no clue as to the amount of solution he employed.

That *contortum* and *recurvum* are sensitive to different degrees is clear, but in the Acidity Table no less than four species lie between these two; it will be understood that to demonstrate a difference in sensitiveness between neighbouring members of that series of six would be a matter of very considerable difficulty.

We must conclude that while the correlation which Paul finds can be

demonstrated for species of markedly different acidities, it is not possible in practice to follow it out in fine detail.

(b) The theory that the toxic action of chalk is due to saturation of the *Sphagnum* acids, and subsequent derangement in metabolism, does not appear probable in view of the various experiments and observations quoted. If the saturation acts directly, it is difficult to see why high concentrations should kill the highly acid species and not those with low acidity. And it is difficult to conceive the mechanism of an indirect action. The *Sphagnum* cannot feel the want of fresh absorbed base at once, for it is capable of living and growing in distilled water for a long time at the expense of previously absorbed base. A *Sphagnum* supplied with a fatal amount of chalk dies promptly, and shows no sign of attempting to manufacture a fresh quantity of acid compound by temporarily increased growth.

The most powerful argument against this view is supplied by the behaviour of *Sphagnum* to salts. These too are capable of saturating the acids, but despite this many of them are supported in high concentrations, although they may be no better nutrients than calcium carbonate. Calcium sulphate was supported in all the species Paul tested, in concentrations up to 2,000 mg. per litre (= 1,400 mg.  $\text{CaCO}_3$ ). The case of calcium chloride is conclusive; it is no better as a food-stuff than the carbonate, and it is supported by *S. medium* up to 966 mg. per litre (= 880 mg.  $\text{CaCO}_3$ ).

If we look for a more natural explanation, the most probable seems to be that it is by altering the reaction of the solution that the carbonate acts. *Sphagnum* can grow best in an acid medium, which it cannot obtain in the presence of chalk. The ability to withstand high concentrations of chalk would then be an ability to withstand strong alkaline reactions. The behaviour of *Sphagnum* to acids and alkalis provides a means to test this hypothesis.

The acids and alkalis chosen—hydrochloric and citric acids, and sodium hydroxide and sodium bicarbonate—could scarcely stimulate growth by supplying mineral nutrients. The results are given in Tables VIII and IX.

TABLE VIII.

| Concentration<br>of alkali. | <i>contortum.</i> |                    | Growth of <i>Sphagnum</i><br><i>recurvum.</i> |                    | <i>rubellum.</i> |                    |
|-----------------------------|-------------------|--------------------|---|--------------------|------------------|--------------------|
|                             | NaOH.             | $\text{NaHCO}_3$ . | NaOH.   | $\text{NaHCO}_3$ . | NaOH.            | $\text{NaHCO}_3$ . |
|                             | %                 | %                  | %   | %                  | %                | %                  |
| N/250                       | 7                 | 7                  | 0   | 13                 | 0                | 0                  |
| N/500                       | 85                | 62                 | 0   | 15                 | 0                | 0                  |
| N/750                       | 90                | 70                 | 0   | 15                 | 0                | 0                  |
| N/1000                      | 63                | 66                 | 0   | 20                 | 0                | 6                  |
| N/2000                      | 70                | 64                 | 62  | 38                 | 20               | 26                 |
| N/3000                      | 100               | 88                 | 70  | 70                 | 26               | 33                 |
| N/5000                      | 80                | 93                 | 93  | 73                 | 106              | 116                |

TABLE IX.

| Concentration<br>of acid. | Growth of <i>Sphagnum</i> |      |                  |      |                  |      |
|---------------------------|---------------------------|------|------------------|------|------------------|------|
|                           | <i>contortum.</i>         |      | <i>recurvum.</i> |      | <i>rubellum.</i> |      |
|                           | HCl.                      | HCl. | HCl.             | HCl. | HCl.             | HCl. |
|                           | %                         | %    | %                | %    | %                | %    |
| N/250                     | 0                         | 49   | 0                | 93   | 0                | 80   |
| N/500                     | 13                        | 100  | 40               | 107  | 0                | 87   |
| N/750                     | 32                        | 124  | 51               | 142  | 33               | 93   |
| N/1000                    | 92                        | 124  | 64               | 127  | 85               | 100  |
| N/2000                    | 118                       | 113  | 118              | 118  | 103              | 106  |
| N/3000                    | 136                       | 142  | 131              | 147  | 127              | 114  |
| N/5000                    | 123                       | 122  | 131              | 147  | 155              | 109  |

The results are a little irregular. We may, however, draw from them the following conclusions :

(a) *contortum* is but little injured by hydroxide of N/500 or less, for *recurvum* the concentration is N/3,000, and for *rubellum* N/5,000. The same holds good for the bicarbonate, except that it is rather more favourable than the hydroxide for *recurvum*. These alkalies act, then, in precisely the same manner as does chalk.

(b) For all three species hydrochloric acid ceases to be harmful at between N/1,000 and N/2,000, citric at N/500. At lower concentrations both acids exercise a very decided stimulating effect on growth.

Taken alone, the results with alkalies may be interpreted in the sense of Paul's hypothesis—in fact, he does quote experiments with alkalies in its support. But in conjunction with the stimulatory effects of acids in low concentrations, they afford good grounds for assuming that the harmful effect of chalk and the alkalies lies in the fact that they deprive the *Sphagnum* of the acid reaction which is beneficial to it.

The method by which *Sphagnum* obtains its supply of nutrient bases entails the liberation of the acids of the salts concerned ; consequently the *Sphagna* are normally bathed in an acid solution. The reaction was at first an accidental accompaniment of another process, but it has now become a necessity for the Moss. Those species living in stations where the salts (and consequently usually also chalk) are scarce require a large quantity of acid compounds ; they are doubly secured from ever encountering an alkaline reaction. Those inhabiting the more favoured low moors, both because they are less acid, and because chalk is more abundant in their environment, have more chance of being subjected to the influence of a neutral or alkaline medium. It naturally follows that the former are more sensitive than the latter. In some such way can we account for the connexion between acidity and sensitiveness. Be that as it may, it would seem that the preference of *Sphagnum* is for an acid reaction, its repugnance, in the case of chalk as in other cases, for an alkaline one.

VI. *SPHAGNUM* AND MINERAL SOLUTIONS.

It remains to consider the fairly widespread opinion that the Bog-mosses are sensitive to high concentrations, as such, of mineral solutions. From experiments quoted it is clear that some salts are much more dangerous than others, but the effect of a complete culture solution has not been tried. In making up a solution, the first difficulty that meets one is the phosphate supply. The extreme toxicity of phosphates has been pointed out by Paul, and emphasized by Haglund. I tried a series of ten phosphates on *S. contortum*, and found it considerably more resistant than the species (*medium*) tested by Paul. It withstood at least 50 mg. of all of them. Further, the toxicity was considerably lessened by the presence of calcium sulphate. In its presence 250 mg. were resisted. In view of this experience a solution of the following constitution was employed :

|                                      |         |
|--------------------------------------|---------|
| Calcium nitrate . . . . .            | 750 mg. |
| Potassium nitrate . . . . .          | 500 mg. |
| Potassium dihyd. phosphate . . . . . | 150 mg. |
| Magnesium sulphate . . . . .         | 400 mg. |
| Potassium chloride . . . . .         | 200 mg. |
| Iron sulphate . . . . .              | trace.  |

The reaction is acid ; but a second set of cultures was tried with the same solution to which just sufficient sodium hydroxide had been added to neutralize the acid. The concentrations used and the results are given in Table X.

TABLE X.

| Concentration. | Growth of <i>Sphagnum</i> |      |                  |      |                  |      |
|----------------|---------------------------|------|------------------|------|------------------|------|
|                | <i>contortum.</i>         |      | <i>recurvum.</i> |      | <i>rubellum.</i> |      |
|                | acid.                     | alk. | acid.            | alk. | acid.            | alk. |
| %              | %                         | %    | %                | %    | %                | %    |
| 0.01           | 118                       | 120  | 170              | 142  | 66               | 63   |
| 0.05           | 138                       | 125  | 147              | 118  | 76               | 43   |
| 0.1            | 135                       | 95   | 149              | 90   | 73               | 20   |
| 0.25           | 112                       | 93   | 110              | 35   | 51               | 33   |
| 0.5            | 60                        | 58   | 70               | 49   | 35               | 27   |

*rubellum* is slightly harmed by even the most dilute acid solutions, and can evidently not support even low salt concentrations. The other two, however, thrive best in stronger solutions. *contortum* is best in 0.05-0.1, *recurvum* in 0.01, though it shows a vigorous growth in the next higher concentrations. When the solution is originally alkaline, the favourable concentrations are lowered in all cases. This again demonstrates very clearly the effect of the alkaline reaction. Although the two more resistant species grow well in solutions of a salt content comparable to that offered in water culture to flowering plants, it does not follow that these conditions will be equally favourable in nature. In my cultures it was seen that after



about two months a vigorous growth of Algae appeared ; in three months the Algae completely covered the Sphagna, so that a continued healthy growth of these was impossible. Haglund, as already stated, made similar observations, and the same thing may be seen frequently in the field with Sphagna growing in ditches.

These experiments yield no data as to the nutrient value of the salts employed. They only show that the less acid Sphagna flourish in quite high concentrations of mineral solutions in artificial cultures.

## VII. CONCLUSIONS.

In addition to the criticism and elucidation of various other points, the chief conclusions which may be drawn from the preceding pages are :

1. There is a variation in acidity and in sensitiveness to chalk between the different species of *Sphagnum*.
2. There is a correlation between degree of acidity and degree of sensitiveness.
3. The connexion between the two is indirect, not direct.
4. The Sphagna thrive in acid solutions: the injurious effect of chalk, and of alkalies in general, is due to the substitution of an alkaline for an acid reaction.
5. Mineral solutions are generally physiologically harmless, but may be ecologically harmful.
6. The Sphagna do actually utilize in growth bases held absorbed by the acid compounds of the cell-walls.

The subject was brought to my notice by Professor Dr. Ludwig Jost, of Strasburg, and to him my most sincere thanks are due for this, and for advice and criticism. To Professor J. W. H. Trail, F.R.S., of Aberdeen, I am indebted for literature references, and to various friends for help, especially in obtaining material.

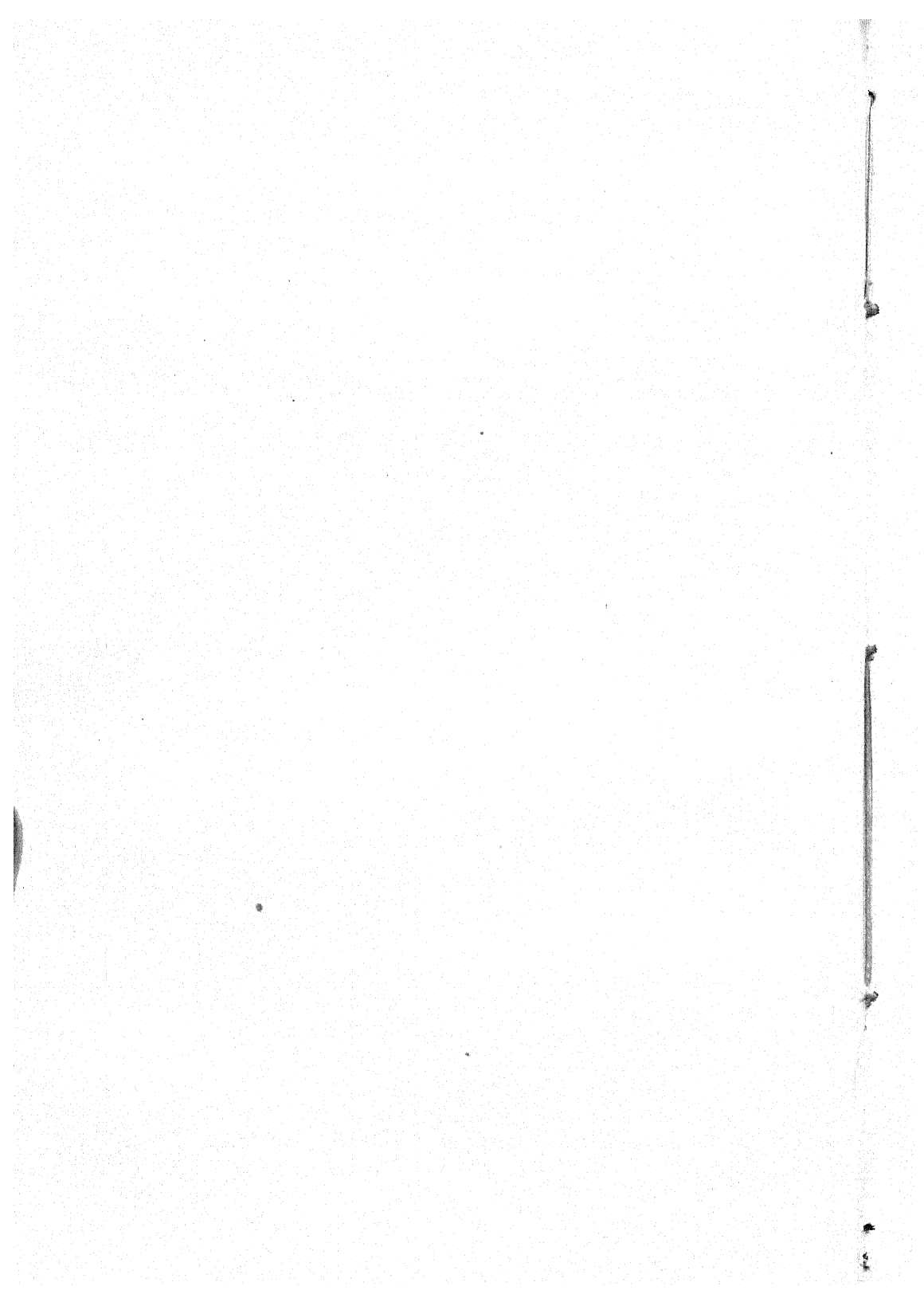
Part of the expense was defrayed by a grant from the Carnegie Trustees, to whom I wish to express my obligations.

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# On the Relation between the Concentration of the Nutrient Solution and the Rate of Growth of Plants in Water Culture.

BY

WALTER STILES.

## INTRODUCTION.

FROM time to time during the last fifty years various writers have published the results of their observations on the effect of the concentration of the nutrient solution on the growth of plants. As a result of these researches from those of Birner and Lucanus<sup>1</sup> onwards, it has become clear that plants grow quite healthily in extremely dilute solutions, but it is not clear that the rate of growth of plants in such solutions is as great as that when higher concentrations are used. Recently Hall, Brenchley, and Underwood<sup>2</sup> have attempted to show that concentration of the nutrient solution influences very greatly the rate of growth of plants. The observations of these writers agree so ill with the conclusions arrived at by other workers, notably by Cameron,<sup>3</sup> that the publication of the results of some experiments on the effect of differences of concentration of the nutrient solution on growth seems justifiable.

## METHODS.

In conducting experiments involving the use of water cultures two main difficulties present themselves. In the first place, plants growing in water cultures under exactly the same conditions are very variable. As evidence of this it is only necessary to cite the results of some work by Brenchley,<sup>4</sup> where the dry weights of a number of plants growing in water culture under exactly similar conditions are given.

<sup>1</sup> Birner und Lucanus : Wasserculturversuche mit Hafer. Landw. Versuchsstat., vol. viii, 1866, pp. 128, 177.

<sup>2</sup> Hall, A. D., Brenchley, W. E., and Underwood, L. M. : The Soil Solution and the Mineral Constituents of the Soil. Phil. Trans. B. 204, 1913, pp. 179-200.

<sup>3</sup> Cameron, F. K. : The Soil Solution. Easton, Pa., 1911.

<sup>4</sup> Brenchley, W. E. : The Influence of Copper Sulphate and Manganese Sulphate upon the Growth of Barley. Annals of Botany, vol. xxiv, 1910, pp. 571-83.

The second difficulty arises from the phenomenon of selective absorption. All ions are not absorbed by the plant at the same rate; the result is that not only the concentrations but the relative proportion of the constituents of the nutrient solution is also changing.

In order to reduce the errors arising from these sources, seeds were used which were of as pure a strain as could be obtained, and which should therefore have yielded plants as alike as possible. The seeds were germinated in clean sand, and young seedlings as much alike as possible were selected.

The plants were grown singly in glass bottles of 1,200 c.c. capacity and were done in sets of ten. The corks used were coated with paraffin, and the solutions were changed every few (five to three) days, except in some instances where inquiry was made into the effect of not changing the nutrient solution. All the cultures in any one series were started on the same day and were also harvested and dried at the same time, so that the results are strictly comparable.

Each plant was dried and weighed separately and the probable error of the mean of each set of ten results calculated, so that an idea of the significance of any differences in dry weight might be obtained.

The nutrient solutions used were of four different concentrations, but the proportions of the contained salts were the same in each. Ordinary 'pure' salts were used and a practically pure distilled water free from copper. The relative concentrations ( $1, \frac{1}{5}, \frac{1}{10}, \frac{1}{20}$ ) were the same as were used by Hall, Brenchley, and Underwood, but the actual proportion of salts was slightly different. The composition of the strongest solution was as follows:

|   |       |       |
|---|-------|-------|
| KNO <sub>3</sub>                                      | 1     | gram. |
| CaSO <sub>4</sub> , 2H <sub>2</sub> O                 | 0.25  | "     |
| MgSO <sub>4</sub> , 7H <sub>2</sub> O                 | 0.25  | "     |
| KH <sub>2</sub> PO <sub>4</sub>                       | 0.25  | "     |
| NaCl  | 0.04  | "     |
| Fe(NO <sub>3</sub> ) <sub>3</sub> , 6H <sub>2</sub> O | 0.04  | "     |
| Water   | 1,000 | c.c.  |

#### THE RESULTS.

A preliminary series was grown during the early months of the year. The plant used was a Danish strain of Rye (Fejøl No. 2), obtained for Professor Priestley by the kind offices of Professor Johannsen. Growth at this time of the year was slow, and consequently when the plants were removed they had not made very much growth. The actual results are as follows. The dry weight of each plant is given.

## Series 1.

Cultures started February 10.

Solutions changed February 13, 17, 20, 24, 28, March 5, 7, 11.

Cultures harvested March 14.

## Concentration of nutrient solution.

| I.                     | 5.      | 10.    | 20.    |
|------------------------|---------|--------|--------|
| gm.                    | gm.     | gm.    | gm.    |
| 0.0439                 | 0.0594  | 0.0316 | 0.0279 |
| 0.0460                 | 0.0604  | 0.0414 | 0.0406 |
| 0.0466                 | 0.0630  | 0.0438 | 0.0463 |
| 0.0493                 | 0.0633  | 0.0484 | 0.0524 |
| 0.0500                 | 0.0678  | 0.0494 | 0.0576 |
| 0.0566                 | 0.0734  | 0.0594 | 0.0657 |
| 0.0590                 | 0.0796  | 0.0604 | 0.0739 |
| 0.0696                 | 0.0834  | 0.0678 | 0.0751 |
| 0.0743                 | 0.0848  | 0.0766 | 0.0814 |
| 0.0992                 | 0.0878  | 0.1122 | 0.0834 |
| Mean                   | 0.05945 | 0.0723 | 0.0591 |
| Probable error of mean | 0.0036  | 0.0023 | 0.0048 |

Reviewing these results after the probable error is taken into consideration, it will be observed that there is not much appreciable difference between the mean dry weights of the plants growing in solutions of different concentration.

A series was grown during the spring in which Barley was employed as the culture plant. Seed of a pure line was used which was kindly sent by Professor Biffen to Professor Priestley. The growth of these plants was much more rapid than that of the Rye grown earlier in the year. The weights of the shoot and root of each plant were taken separately. The results of this series are as follows:

## Series 2.

Cultures started April 28.

Solutions changed May 5, 8, 13, 18, 22, 26, 29, June 2.

Cultures harvested June 6.

## Concentration = 1.

| Shoot.  | Root. | Total.             |
|---|-------|--------------------|
| gm.   | gm.   | gm.                |
| 0.319   | 0.078 | 0.397              |
| 0.341   | 0.083 | 0.424              |
| 0.369   | 0.096 | 0.465              |
| 0.387   | 0.106 | 0.493              |
| 0.476   | 0.128 | 0.605 <sup>1</sup> |
| 0.531   | 0.122 | 0.653              |
| 0.576   | 0.140 | 0.716              |
| 0.592   | 0.146 | 0.739              |
| 0.586   | 0.155 | 0.741              |
| 0.838   | 0.208 | 1.046              |
| Mean  | 0.502 | 0.126              |
| Probable error of mean  |       | 0.041              |
| $\frac{\text{Dry weight of shoot}}{\text{Dry weight of root}} = 4.0.$ |       |                    |

<sup>1</sup> In all cases the plants were weighed to a tenth of a milligram, but the numbers are here given correct to the third decimal place.

Concentration =  $\frac{1}{5}$ .

| <i>Shoot.</i> | <i>Root.</i> | <i>Total.</i>      |
|---------------|--------------|--------------------|
| <i>gram.</i>  | <i>gram.</i> | <i>gram.</i>       |
| 0.239         | 0.082        | 0.320 <sup>1</sup> |
| 0.359         | 0.097        | 0.456              |
| 0.377         | 0.120        | 0.497              |
| 0.412         | 0.120        | 0.532              |
| 0.427         | 0.111        | 0.538              |
| 0.476         | 0.125        | 0.601              |
| 0.474         | 0.131        | 0.605              |
| 0.547         | 0.129        | 0.676              |
| 0.546         | 0.158        | 0.704              |
| 0.785         | 0.204        | 0.989              |
| Mean          | 0.489        | 0.622              |

Probable error of mean

$$\frac{\text{Dry weight of shoot}}{\text{Dry weight of root}} = 3.7.$$

Concentration =  $\frac{1}{10}$ .

| <i>Shoot.</i> | <i>Root.</i> | <i>Total.</i> |
|---------------|--------------|---------------|
| <i>gram.</i>  | <i>gram.</i> | <i>gram.</i>  |
| 0.188         | 0.080        | 0.268         |
| 0.315         | 0.080        | 0.395         |
| 0.313         | 0.099        | 0.412         |
| 0.382         | 0.101        | 0.483         |
| 0.361         | 0.136        | 0.497         |
| 0.425         | 0.112        | 0.537         |
| 0.415         | 0.140        | 0.554         |
| 0.466         | 0.124        | 0.590         |
| 0.569         | 0.151        | 0.721         |
| 0.611         | 0.198        | 0.809         |
| Mean          | 0.429        | 0.555         |

Probable error of mean

$$\frac{\text{Dry weight of shoot}}{\text{Dry weight of root}} = 3.4.$$

Concentration =  $\frac{1}{20}$ .

| <i>Shoot.</i> | <i>Root.</i> | <i>Total.</i> |
|---------------|--------------|---------------|
| <i>gram.</i>  | <i>gram.</i> | <i>gram.</i>  |
| 0.243         | 0.096        | 0.339         |
| 0.279         | 0.063        | 0.342         |
| 0.312         | 0.084        | 0.396         |
| 0.342         | 0.113        | 0.455         |
| 0.357         | 0.113        | 0.470         |
| 0.362         | 0.116        | 0.478         |
| 0.370         | 0.143        | 0.513         |
| 0.394         | 0.123        | 0.517         |
| 0.396         | 0.135        | 0.531         |
| 0.405         | 0.133        | 0.538         |
| Mean          | 0.354        | 0.471         |

Probable error of mean

$$\frac{\text{Dry weight of shoot}}{\text{Dry weight of root}} = 3.0.$$

<sup>1</sup> The plants whose dry weights are shown in italics were of an entirely different form from the rest. They were smaller, with finer leaves, of a much bushier and much less elongated habit, and of a much darker green. All the other plants were so similar in form that the three exceptions noted in the tables are obviously not in the pure line. Their dry weights have therefore not been considered in calculating the mean or the probable error.



Summarizing these results and taking the probable error into account the following numbers are obtained :

| <i>Concentration of<br/>nutrient solution.</i> | <i>Dry weight in grm.<br/>Highest and lowest<br/>numbers.</i> |       |
|--|---|-------|
| 1  | 0.669   | 0.587 |
| $\frac{1}{5}$                                  | 0.657   | 0.587 |
| $\frac{1}{10}$                                 | 0.585   | 0.525 |
| $\frac{1}{20}$                                 | 0.486   | 0.456 |

Thus the only mean dry weight which differs from the others by an amount exceeding the probable error is that of the cultures growing in the dilutest solution, and even here the difference is not very great.

As the concentration of this particular solution is less in all essential things, except nitrate and iron, than the lowest strength of nutrient solution used by Hall, Brenchley, and Underwood, it is surprising that the difference in the dry weight between the cultures grown in this solution and those grown in the highest strengths should be so small. The numbers obtained by Hall, Brenchley, and Underwood are as follows :

| <i>Concentration of<br/>nutrient solution.</i> | <i>Dry weight.</i> |
|--|--------------------|
| 1  | 0.420              |
| $\frac{1}{5}$                                  | 0.244              |
| $\frac{1}{10}$                                 | 0.108              |
| $\frac{1}{20}$                                 | 0.068              |

In order to determine whether an infrequent changing of the nutrient solutions influences the result, two further series of Barley cultures were grown in solutions which were seldom changed. In all other respects these cultures were conducted similarly to the others. They were done in sets of ten and the probable error of the mean dry weight of each set calculated. The numbers already given serve to illustrate the variation in dry weight obtained within one set, and so individual results are not given in the following tables. The results are as follows :

### *Series 3.*

Cultures started April 30.

Solutions changed May 11, 21.

Cultures harvested June 9.

| <i>Concentration<br/>of solution.</i> | <i>Dry weight<br/>of shoots.<br/>grm.</i> | <i>Dry weight<br/>of roots.<br/>grm.</i> | <i>Total dry<br/>weight.<br/>grm.</i> | <i>Probable<br/>error.<br/>grm.</i> | <i>Shoot<br/>root.</i> |
|---------------------------------------|---|--|---------------------------------------|-------------------------------------|------------------------|
| 1                                     | 0.332                                     | 0.110                                    | 0.442                                 | 0.022                               | 3.0                    |
| $\frac{1}{5}$                         | 0.3485                                    | 0.1124                                   | 0.461                                 | 0.019                               | 3.1                    |
| $\frac{1}{10}$                        | 0.3437                                    | 0.1046                                   | 0.448                                 | 0.012                               | 3.3                    |
| $\frac{1}{20}$                        | 0.189                                     | 0.077                                    | 0.266                                 | 0.011                               | 2.5                    |

*Series 4.*

Cultures started May 1.

Solutions changed May 25.

Cultures harvested June 12.

| <i>Concentration<br/>of solution.</i> | <i>Dry weight<br/>of shoots.</i> | <i>Dry weight<br/>of roots.</i> | <i>Total dry<br/>weight.</i> | <i>Probable<br/>error.</i> | <i>Shoot<br/>root.</i> |
|---------------------------------------|----------------------------------|---------------------------------|------------------------------|----------------------------|------------------------|
|                                       | <i>gm.</i>                       | <i>gm.</i>                      | <i>gm.</i>                   | <i>gm.</i>                 |                        |
| 1                                     | 0.227                            | 0.085                           | 0.312                        | 0.017                      | 2.7                    |
| $\frac{1}{2}$                         | 0.325                            | 0.103                           | 0.428                        | 0.021                      | 3.2                    |
| $\frac{1}{4}$                         | 0.377                            | 0.123                           | 0.500                        | 0.020                      | 3.1                    |
| $\frac{1}{8}$                         | 0.2826                           | 0.1287                          | 0.411                        | 0.018                      | 2.2                    |

## DISCUSSION OF RESULTS.

The results recorded in the preceding section of this paper indicate that if the nutrient solutions in water-culture experiments are changed frequently, so as to maintain more nearly a constant composition of the solution, the concentration of the solution may vary considerably without producing much effect on the rate of growth of the cultures as measured by the dry matter produced within a given time. Below a certain concentration, however, there seems to be an indication that the rate of growth becomes less, although this falling off is not very marked in the lowest concentration employed in these experiments, and not nearly so marked as the falling off in the growth recorded by Hall, Brenchley, and Underwood for the same species growing in rather similar concentration. Indeed, the results concerning Rye and Barley recorded in the present paper are exactly described by Cameron when he states with regard to water-culture experiments with Wheat, 'that if a given ratio of mineral nutrients be maintained, relatively small effect is produced on the growing plants by varying the concentration over a wide range'.<sup>1</sup> The recently published work of Tottingham<sup>2</sup> indicates the same conclusion. It should be stated that the plants which had produced the least dry matter were quite healthy plants and showed no sign of weakening.

Indeed, of the 160 plants grown none died nor showed any sign of lack of vigour when the cultures were stopped.

It will be observed that when the nutrient solutions remain unchanged a marked depression of the rate of growth occurs. This can scarcely be due to lack of salt in the strongest nutrient solutions, although this cause may be operative in the solutions of weaker concentration. It might be caused by the harmful effect of excreta from the plant, but at present the existence of such toxic excreta cannot be regarded as definitely established. It might more probably be explained by the absorption of

<sup>1</sup> The Soil Solution, p. 70.

<sup>2</sup> Tottingham, W. E.: A Quantitative Chemical and Physiological Study of Nutrient Solutions for Plant Cultures. *Physiol. Researches*, vol. i, 1914, pp. 133-245.

different ions at different rates which would result after a time in an alteration of the relative proportions of the different substances in the solution. The necessity for a definite balance between the substances in a nutrient solution has been emphasized by many workers recently. The effect of this selective absorption would be extremely difficult to foretell, as it would probably produce different results in solutions of different concentrations. In the strongest solutions, however, the toxic properties of the substance in excess would probably be most marked, while in the weakest solution a starvation effect owing to exhaustion of some particular salt or ion might result. In any case it would appear to be essential in many water-culture experiments to renew the culture solutions at frequent intervals, and possibly to use culture jars or bottles of large capacity.

One point which may be worth mentioning is that of the ratio of the dry weight of the shoot to that of the root. It would appear that with decreasing concentration of the solution the growth of the shoot is affected much more than that of the root, a fact which is also indicated by Hall, Brenchley, and Underwood's figures.<sup>1</sup> When the culture-solutions are not changed frequently, the growth of the shoots is again affected more than that of the roots.

It seems necessary to lay emphasis on the extreme variability of plants growing in water-cultures, particularly as regards their dry weight. The numbers given by Brenchley, already referred to in this paper, and some given by Hall, Brenchley, and Underwood,<sup>2</sup> make it quite clear that in order to obtain definite results by the water-culture method it is essential to use a fairly large number of plants, and to weigh the dry matter of each plant separately and calculate the probable error.

Only by this means can an indication be obtained as to whether any difference is significant. By reference to the figures in this paper it will be seen that working with sets of ten plants under the same conditions does not allow of the measurement of moderately small differences even when a pure line of seed is used. Hall, Brenchley, and Underwood's cultures were only grown in duplicate, and this may account for the differences between their results and those of other observers.

What bearing the results of experiments with water-cultures can have on the question of the soil solution it is difficult, and would indeed be premature, to say. To argue from a comparatively simple medium, such as a nutrient solution of mineral salts, to a complex structure like the soil, is indeed a risky thing to do in the present state of our knowledge. It seems, however, safe to say that the present experiments, like those of most other observers, support Cameron's contention that the soil solution, dilute as he supposes it to be, is yet quite concentrated enough to support vegetation. In this connexion it is interesting to compare the quantities of

<sup>1</sup> Hall, Brenchley, and Underwood: *l. c.*, pp. 191, 193.

<sup>2</sup> *l. c.*, pp. 191, 195.

potassium and phosphate in the various solutions used in these experiments with the quantities found by Cameron to be present in the soil solution.

| <i>Parts per<br/>10<sup>6</sup> of</i> | <i>Concentration of solution.</i> |               |                |                | <i>Soil solution<br/>according to<br/>Cameron.</i> |
|--|-----------------------------------|---------------|----------------|----------------|--|
|  | 1                                 | $\frac{1}{5}$ | $\frac{1}{10}$ | $\frac{1}{20}$ |  |
| K <sub>2</sub> O                       | 552                               | 110           | 55             | 28             | about 28   |
| P <sub>2</sub> O <sub>5</sub>          | 131                               | 26            | 13             | 6.5            | about 7  |

Thus the weakest solution when first put in the culture jars was of the same strength in regard to potash and phosphate as that of the soil solution. This probably means that its average strength during the time it was in the culture bottles was somewhat less; and although the plants grown in this dilutest solution produced somewhat less dry matter than those in higher strengths, the difference was not great and the plants were perfectly healthy.

Finally, it should be pointed out that such results as those here recorded cannot be regarded as in any way general. Although all the plants in one series were grown under apparently exactly the same conditions, yet it is possible that under a different set of conditions a different result might be obtained. Again, different species might show different effects in regard to concentration of the nutrient solution. It must be left for future work to deal with these questions.

#### SUMMARY.

1. The variation over a fairly wide range of the concentration of the nutrient solution of Rye and Barley growing in water culture produces relatively little effect on the amount of dry matter produced. Below a certain concentration there appears to be a definite falling off in the rate of growth.
2. The concentration of the soil solution as estimated by Cameron, low as it is, is yet high enough to produce healthy plants.
3. Frequent changing of the nutrient solution of water cultures produces decidedly better growth of the plants.
4. It is necessary to calculate the probable error of the results obtained in experiments with water-cultures in order to determine the significance of differences between results from different sets of cultures.

BOTANY DEPARTMENT,  
THE UNIVERSITY, LEEDS.  
*June 25, 1914.*

# Obligate Symbiosis in *Calluna vulgaris*.<sup>1</sup>

BY

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With Plate VI and four Figures in the Text.

## INTRODUCTORY.

AS an important member of the moorland flora of Northern Europe, *Calluna vulgaris* is commonly associated with soils of a definite type, especially when it occurs as the dominant or sub-dominant species of moorland associations. As such, it is especially characteristic of dry heath soils, deficient in lime and often acid in reaction.

So obviously is this the case, that the current hypothesis to explain the edaphic relations of this and other calcifuge ericaceous species assumes that their local dominance connotes soil conditions inimical to the growth of other plants.

This hypothesis, however, did not adequately explain the success of small *Calluna* communities in the special case selected,<sup>2</sup> and a close scrutiny of ecological records revealed similar inconsistencies elsewhere.

Experimental evidence has already been brought forward indicating that the 'lime-shy' habit is not a direct reaction to excess of calcium carbonate *as such* in the soil, but is associated with correlated peculiarities of calcareous soils (1). It seemed possible that detailed experimental investigation of a special case might throw light on the significance of the 'calcifuge' habit in general.

A detailed record of the soil conditions found in the special case referred to above has already been published (2).

The observations recorded in this earlier paper, taken in conjunction with the results of water-culture experiments on seedlings of *Calluna* extending over a year, served to strengthen the impression that the soil preferences of the plant are probably intimately connected with the biological relations of the roots with one or more micro-organisms.

<sup>1</sup> Thesis approved for the degree of Doctor of Science in the University of London.

<sup>2</sup> The investigation of which this paper gives an account was undertaken as the result of an inquiry into the precise ecological conditions—edaphic and biological—which are associated with the presence of small well-defined communities of the Common Ling (*Calluna vulgaris*) in a restricted area of the Wiltshire Downs.

Subsequent experimental work led to the following conclusions, the data in support of which have been recorded (3):

1. Seeds of *Calluna vulgaris* may be sterilized, and seedlings germinated in a sterile condition, i. e. free from fungal or bacterial infection.

2. In seeds removed with aseptic precautions from unopened fruits, the embryo and endosperm are sterile; the testa is infected with a Fungus identical with the mycorrhizal Fungus of the root.

3. Germination and the early stages of growth of sterile seedlings are normal, but, in the absence of infection, arrest of root formation occurs, with subsequent inhibition of growth.

4. Infection of the primary root of the seedling takes place subsequent to germination, by a growth of mycelium from the seed-coat, the latter being infected while still in the ovary.

5. Pot cultures in soils, favourable and unfavourable respectively to the growth of the plant in the field, demonstrate that *Calluna* grows *normally* in the former, *abnormally* in the latter. Abnormality of growth is exhibited in:

(a) reduced germination capacity; (b) retarded germination; (c) arrest of seedling root and curvatures of growing region; (d) arrest of shoot; (e) small size and red coloration of leaves.

6. Intimately connected with these abnormalities is the presence of colonies of Bacteria on the roots, especially round the tips. These Bacteria are to be regarded either as pathogenic agents, or as indicators of soil conditions unfavourable to the Fungus or to the plant. The presence of bacterial colonies is directly correlated with the abnormal growth displayed by the roots, but evidence is not conclusive that they are the immediate *cause* of this condition.

7. The evidence available points therefore to the conclusion that the relation between the plant and its mycorrhizal Fungus is an obligate one. Successful growth is bound up with infection of the roots at an early stage by the Fungus, and with the subsequent healthy growth of the latter. The soil preferences exhibited by the plant depend on the maintenance of a biological balance between the roots and the constituents of the microflora which beset them.

Assuming these conclusions to be valid, it is apparent that before any special case involving the soil preferences of the plant can be attacked directly—before, indeed, such a problem can be clearly formulated—the ground must be cleared by an investigation into the precise relations existing between the plant and the Fungus which plays such an important part in its life-history.

The work recorded in the present paper was commenced with this end in view. Owing to the somewhat unexpected nature of the results, it has been carried out as an independent research.

The facts established supply the data requisite for a direct attack upon the original ecological problem ; they throw further light on the rather vexed question of the significance of mycorrhiza, and they have demonstrated for *Calluna*, and probably for most ericaceous species, an unsuspected symbiotic relation, in some respects unique among flowering plants.

The record of the results of the present investigation, since it is concerned more especially with the isolation and biology of the mycorrhizal Fungus of *Calluna*, is prefaced by a brief historical review of previous work on endotrophic mycorrhiza.

#### MYCORRHIZA—HISTORICAL.

The peculiar association of the vegetative stage of a Fungus with the roots of the higher plants, known by this name, has been familiar to botanists since the middle of the last century, and although many cases have since been carefully investigated, especially from the cytological point of view, comparatively little is yet known with certainty of the bionomics of the relationship, and still less of the life-histories and systematic position of the Fungi concerned.

Early observers were usually content to record the presence of hyphae in or upon the roots of various plants without attempting to investigate the exact relations between the two organisms.

In the early part of the nineteenth century the presence of mycelium in plant tissues was recorded by Schleiden, and in 1882 Kamienski drew attention to the external investment of hyphae on the roots of *Monotropa Hypopitys* (4).

In 1887 a great impetus was given to research by Frank, who first made use of the term *mycorrhiza* and formulated a definite theory of symbiosis, the possibility of which had already been suggested by Pfeffer, Kamienski, Treub, and Goebel.

Frank's observations were made on a large number of plants, and he distinguished two forms of union: *ectotrophic mycorrhiza*, in which the Fungus forms an external investment on the root but does not penetrate the cells—especially characteristic of many forest trees; and *endotrophic mycorrhiza*, in which the cells of the plant are actually invaded by fungal hyphae.

Based on experimental study of ectotrophic mycorrhiza in forest trees, Frank formulated his well-known theory of the symbiotic rôle of the Fungus; namely, that root-hairs are frequently absent and are replaced functionally by fungal hyphae, which are responsible for the absorption by the plant of mineral salts or organic nitrogen compounds from the soil—a debt repaid by the transference of carbohydrates from plant to Fungus.

Subsequently, a similar function was claimed for the Fungus of endotrophic mycorrhiza, the absorption of nitrogen compounds from the Fungus involving, in this case, their digestion by the cells of the plant (5).



Frank and his pupil Schlicht drew attention to the widespread occurrence of endotrophic mycorrhiza, especially in the Natural Orders Orchidaceae, Epacridaceae, and Ericaceae.

In the case of Ericaceae, Frank described and figured the very fine hair-like roots of certain heath plants, the cells of which are constantly filled with fungal hyphae (6).

In such roots he emphasizes the complete absence of root-hairs, the disappearance or reduction in amount of cortical tissue, and observed the 'epidermal' layer of large cells filled with 'knot-like' masses of mycelium, branches from which penetrate the external walls.

Frank recognized the possible existence of more specialized relations between Fungus and flowering plant in the case of certain groups, such as the Ericaceae and Orchidaceae, but pointed out that comparative cultures of *infected* and *uninfected* plants of this type were not yet available for discussion.

The conclusions of Frank as to the rôle of the Fungus in ectotrophic mycorrhiza were challenged by Sarauw (7) and by Möller (8), the latter of whom pointed out that ectotrophic mycorrhiza was often well developed in soils poor in humus.

It was also shown subsequently that root-hairs were not uncommonly formed by plants possessing ectotrophic mycorrhiza, and Von Tubeuf (9) demonstrated that the endotrophic type was often characteristically developed in forest trees.

On the whole, since Frank's time, views as to the significance of ectotrophic mycorrhiza have diverged from his theory without entirely abandoning it.

Subsequent to the researches of Frank, the endotrophic forms attracted more attention. Among a number of publications dealing more especially with their cytology and general significance may be mentioned those of Groom (10), Thomas (11), Janse (12), Magnus (13), Shibata (14), and Peklo (15). The first of these authors agreed substantially with the view of Schlicht and recognized a series of transition forms leading from the condition observed in Ericaceae—which, in his view, approached the ectotrophic type present in forest trees—to the highly specialized relations found in *Thismia Aseroë*.

In 1900 Stahl published his well-known work on the comparative biology of autotrophic and mycotrophic plants (16).

He assigned to the fungal partner of mycotrophic plants the rôle of obtaining mineral salts from the soil, and pointed out that such salts are especially valuable to plants which, because they grow in humus, or for other reasons (e.g. slow transpiration), are unable to absorb water with sufficient rapidity to satisfy their requirements for mineral salts.

The experimental work on which Stahl based his conclusions would

seem to be open to criticism in the light of recent researches, and is further discussed at the end of this paper (p. 125).

In recent years the mycorrhiza of the Orchids has attracted attention owing to the abundance of the endophyte in the tissues, and to its very characteristic relations with the cells of the plant.

The researches of Bernard mark an epoch in the knowledge of endotrophic mycorrhiza and of its biological significance. His discoveries were not only of great theoretical interest but are of some potential value to Orchid growers.

It had long been known to horticulturists that the seeds of Orchids—especially of certain genera, e. g. *Odontoglossum* and *Vanda*—are extremely difficult to germinate.

Working with a number of Orchid species, Bernard found that it was impossible to germinate seeds removed under aseptic conditions from sterilized capsules. Some degree of development usually took place, but except in rare cases—e. g. *Bletilla hyacinthina*—the embryo did not reach an advanced stage. In no case, in his cultures, did development of the seedling reach the stage of root formation, unless injection from the substratum took place.

The next step in the investigation was the isolation of the mycorrhizal Fungus; seed cultures were inoculated from a pure culture of the Fungus so obtained, and normal Orchid seedlings were raised successfully by this means.

Various transition stages with regard to the degree of dependence of the plant upon the Fungus were observed by Bernard.

In *Bletilla hyacinthina*, a relatively unspecialized type, uninfected seeds germinate and the seedlings form several leaves, i. e. the plantlet reaches a comparatively advanced stage, but is unable to develop roots without infection. In other Orchids, development of the embryo is arrested at a much earlier stage.

According to Bernard, the degree of specificity between plant and Fungus is variable: thus, using species of *Cattleya* and *Cypripedium*, he found that the Fungus isolated from a species of the one genus could be used successfully for inoculation of the other, but in the case of seeds of species of *Phalaenopsis* and *Odontoglossum*, which under normal conditions are difficult to germinate, the Fungus was found to be specific to the plant. It was determined further that Orchid species differ in respect of their ability to resist invasion by the mycorrhizal Fungus from another species. Thus, in the case of a species of *Phalaenopsis*, infection by the Fungus of *Cattleya* sp. killed the seeds outright—the plant cells made no attempt to resist the attack; in another species, infection took place, followed by digestion of the Fungus by the cells of the embryo and subsequent arrest of development.

In the Orchids, therefore, the symbiotic relation between plant and micro-organism is an obligate one, and has resulted in a high degree of

dependence on the part of the former. The Fungus has been isolated, grown in pure culture outside the plant, and used with success for the reinoculation of sterile seeds (17).

A theory of tuberization, as a general consequence of infection by mycelium, was elaborated by Bernard as a result of his earlier researches (18).

In 1909 Burgeff published a monograph on the root Fungi of the Orchids, recording researches in the course of which he repeated the experiments and confirmed the results of Bernard, and made further contributions to our knowledge of the metabolism and physiology of the endophytes (19).

The conclusions of Burgeff differ somewhat from those of Bernard with respect to the degree of degeneration shown by the Fungus when grown outside the plant, as tested by subsequent inoculation.

According to the latter author, the longer the Fungus is grown as an independent organism outside the plant, the more markedly does it lose its power of causing germination and inducing root formation. Burgeff, on the contrary, claims that one of his Fungi, after growing for twenty-six months on artificial media, had the same capacity for effecting germination as when first isolated from the plant.

For purposes of comparison with the more specialized types, Gallaud (20) investigated a large number of Phanerogams, the roots of which contain an endophytic mycelium, but in which the relations between plant and hyphae are apparently more simple and unspecialized than in the case of the Orchids.

The researches of Gallaud appear to be of special importance in regard to the evolution of the more specialized types of root symbiosis, and some of the facts are discussed from this point of view in a subsequent part of this paper (p. 127).

It is of interest to note that, when selecting endotrophic types for examination, Gallaud rejected the members of Ericaceae for the purpose, as possessing a mycorrhiza more nearly related to the ectotrophic forms.

So far from this being the case, I hope to demonstrate in the following pages that the conditions existing in *Calluna vulgaris* (and probably common to all ericaceous species) connote a wider distribution of the endophyte in the tissues than has been recorded hitherto for any mycorrhizal Fungus, involving in some respects the most highly specialized relation between the two symbionts that has been yet described for a flowering plant and a Fungus.

#### *The Fungi concerned in Endophytic Mycorrhiza. Historical.*

Up to the present, the Orchids have provided the only case in which absolute proof of the identity of the endophytic Fungus has been established by the successful inoculation of sterile seeds or seedlings from a pure culture (p. 101).

With this exception, in spite of the large and constantly increasing number of plants which are known to possess an endotrophic Fungus in the root tissues, nothing is known with certainty of the systematic position of the Fungi concerned.

Many workers have endeavoured to isolate the Fungus concerned in endotrophic mycorrhiza, and the history of these efforts is not without interest.

The task of isolating the Fungus from pieces of Orchid root was essayed by Wahrlich (1886) (21), Chodat and Lendner (1898) (22), and Bernard (1903) (23), and in all three cases resulted in the extraction of a species of *Fusarium*, which was believed for a time to be the true endophyte.

Gallaud (1905) (20), in a series of researches extending over several years, worked on thirty-five species of flowering plants of widely separated affinities. From the roots of thirty of these he isolated with great regularity a species of *Fusarium*, which he obtained also but with rather less regularity from the roots of four of the remaining species.

His cultures showed that, in addition to *Fusarium*, a number of other Fungi were constantly present on washed roots; among the more common genera were *Mortierella*, *Trachoderma*, *Cephalospermum*, and *Gliocladium*, with which are constantly associated species of *Alternaria*, *Acrostagmus*, &c.

Inoculation experiments showed that *Fusarium* and the other species named were not the true endophytes, and established the fact that the species obtained in this way are constituents of the mycelial flora habitually associated with roots growing in ordinary soils.

Gallaud concluded that it was impossible to extract the endophyte directly from the roots, and believed the difficulty to arise in some degree from the alteration or partial digestion undergone by the Fungus in the cells of the plant, and its subsequent inability to grow out of the cells.

Bernard, in 1903 and succeeding years, isolated fungal species from the roots of various Orchids; using a pure culture of the appropriate Fungus, and sterile seeds removed from unopened capsules with aseptic precautions, he was able—for the first time—to effect the synthesis of Orchid plant and Fungus, and thus to induce successful germination of the former. He referred these Fungi provisionally to the genus *Oospora* (24).

In the course of subsequent researches (1905, 1906) (25) *Rhizoctonia* was suggested as the nearest generic ally among free-living Fungi for species isolated from *Phalaenopsis*, *Odontoglossum*, and *Cattleya*.

Burgeff holds that the endophytic Fungi of the Orchids are to be regarded as forming a group morphologically and physiologically distinct, for which he suggests the generic name of *Orcheomyces*, leaving the correct systematic position of the genus an open question (14).

Ternetz (26), working at the root Fungi of Ericaceae, isolated pycnidia-

producing Fungi from the roots of various ericaceous species (of which *Calluna vulgaris* appears to have been one) and referred them to the genus *Phoma*. Complete proof of the identity of these Fungi with the species endophytic in roots of the respective plant-species was unfortunately not possible, since sterile seedlings were not obtained. Incidentally, this author recognized the possibility of seed-coat infection in Ericaceae, but did not investigate it further.

It was claimed that five of these species of *Phoma* in pure culture fixed nitrogen from the air, although in very different degrees; in no case was combined nitrogen found necessary for their development. Appreciable nitrogen fixation from the air has been claimed also by this author for *Aspergillus niger* and for *Penicillium glaucum*, and this result was corroborated by Froelich (27) in 1907 for the same species. On the other hand, a negative result is reported for the same and additional species by Wino-gradski (28), Koch (29), Czapek (30), &c., and more recently by Goddard for a large number of soil Fungi (31).

The fixation of free nitrogen from the air has been claimed also for the endotrophic Fungus of *Podocarpus* (32), and is suggested, but not established, for the Fungus which infests the seed and vegetative parts of *Lolium temulentum* (33). There is no evidence for fixation of atmospheric nitrogen by the Fungi concerned in the root mycorrhiza of Orchids, and attempts to cultivate them on nitrogen-free substrata have not been successful (19).

The whole question of the possibility of fixation of free nitrogen by Fungi growing on nitrogen-free media, as determined by direct estimation of the combined nitrogen present at the end of the experiment, is still in an unsatisfactory condition, and, in spite of the positive results claimed by a number of observers, the balance of evidence appears to be on the negative rather than on the positive side.

The sources of error are obvious. The possibility of working with impure cultures, the difficulty of obtaining a substratum free from traces of nitrogen, and the small quantities of nitrogen fixation reported by most observers who claim a positive result, together with the comparatively large margin of experimental error, demand greater uniformity of results before ability to fix free nitrogen can be claimed as a general property of Fungi in the same or even in less degree than has been finally established and placed on a sure scientific basis for a number of Bacteria.

#### THE PRESENT INVESTIGATION.

The purpose of the investigation described in the following pages is as follows:

1. To confirm and extend the conclusions already reached with respect to the inability of *Calluna* seedlings to form roots unless infected at an early stage by a specific Fungus identical with that present in the cells of the root,

and to demonstrate infection of the seeds by this Fungus while still enclosed in the fruit (p. 98).

2. To investigate the possibility of replacing the stimulus resulting from fungal infection of the seedling by small amounts of various organic substances supplied to sterile seedlings (grown under aseptic conditions), in addition to the requisite mineral salts.

3. To determine the source of infection of the ovary tissues (p. 98).

4. To isolate the mycorrhizal Fungus and establish its identity by successful inoculation of sterile seedlings.

5. To investigate the life-history of the Fungus when grown in pure culture as an independent organism outside the plant.

1. *Dependence of root formation on infection.* The conclusions already reached with regard to inability of sterile seedlings to form a root-system have been confirmed.

If due precautions are observed, seeds can be sterilized without injury to the embryo by washing in 1 per cent. corrosive sublimate solution. Complete sterilization is not easy to effect, and the margin of safety is a narrow one, owing no doubt to the delicacy of the testa and the fact that infection of the cells of the seed-coat is more extensive and deep-seated than is the case with air-infected seeds.

If seeds were left a few seconds too long in the sterilizing solution, the embryo was killed outright, germination was delayed, or the seedlings which germinated showed complete chlorosis.

After sterilization by this method and repeated washings in distilled water, seeds were sown by means of a sterile pipette on agar plates, and kept under dust-free conditions in a closed germinator.

The agar medium on which they were sown contained dextrose and peptone in addition to mineral salts, in order to facilitate the growth of micro-organisms if present. By this means a number of plates of seedlings were obtained which remained entirely free from infection by micro-organisms.

As an additional test of sterility, seedlings were transferred singly to tubes of glucose broth and other media, and kept under observation for three weeks. All the tubes remained sterile.

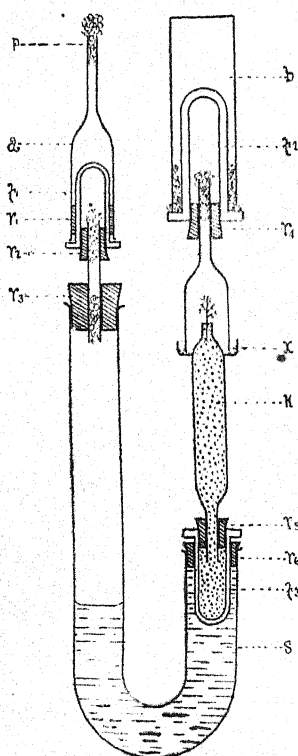
The evidence is conclusive therefore that the embryo and endosperm of *Calluna* seeds are free from infection, and that, by adequate sterilization, seedlings can be obtained free from infection by micro-organisms. Sterile seedlings obtained in this way were planted out in sand and in agar, in a series of cultures extending over several years.

The tubes originally used for sterile sand cultures are described and figured elsewhere (3).

In order to eliminate imperfect aeration as a possible factor in the non-production of roots, a special apparatus was designed for use in subsequent

cultures, which allowed air to be drawn through the sand in the tubes periodically (Text-fig. 1).

These tubes proved satisfactory and remained sterile for several months, but gave no better results than the non-aerated sand cultures. In



TEXT-FIG. 1. Apparatus for growing sterile sand cultures.  $f_1$ - $f_3$  = Massen filter candles;  $r_1$ - $r_6$  = rubber corks;  $a$  = glass cap for attachment to aspirator;  $b$  = glass cap to exclude dust;  $k$  = sand;  $p$  = cotton-wool plug;  $s$  = nutrient solution;  $x$  = wax joint.

no case was a root-system developed. Sterile seedlings from these sand cultures resembled those from agar cultures (Text-fig. 2).

Unsterilized controls cultivated for several months in these tubes grew slowly but developed a normal root-system (Pl. VI, Fig. 10).

For agar cultures, sterile seedlings were transferred to tubes containing a solution previously used with success for water cultures,<sup>2</sup> made up with 0.12 per cent. agar. These cultures have an advantage over those grown in sand in that infection of the medium by micro-organisms is at once evident. Such tubes have been maintained in a sterile condition for ten months.

The seedlings usually formed a few leaves, chlorotic or reddish in colour, but did not develop roots, although they remained in a turgid condition and apparently alive for five to six months (Text-fig. 2).

The cultivation of unsterilized controls in agar presents some difficulty owing to the vigorous development of the epiphytic micro-flora associated with the roots. Otherwise the growth of such seedlings is normal, and comparable with that of those grown in soil, e.g. the shoot-system of such a seedling had reached a height of 5 cm. four months after planting.

<sup>1</sup> The water-culture solution known for the sake of brevity as Solution A was one in which unsterilized seedlings grow vigorously (3, p. 60). It formed the basis of all media used in experimental cultures. The composition is as follows:

|  |        |       |
|--|--------|-------|
| Potassium nitrate ( $\text{KNO}_3$ )                         | 1.0    | gram. |
| Magnesium sulphate ( $\text{MgSO}_4$ )                       | 0.4    | gram. |
| Calcium sulphate ( $\text{CaSO}_4$ )                         | 0.5    | gram. |
| Calcium monophosphate ( $\text{CaH}_4\text{P}_2\text{O}_8$ ) | 0.5    | gram. |
| Sodium chloride ( $\text{NaCl}$ )                            | 0.5    | gram. |
| Ferric chloride  | Trace. |       |
| Water  | 2,000  | c.c.  |

(Acidity: 0.012 normal.)

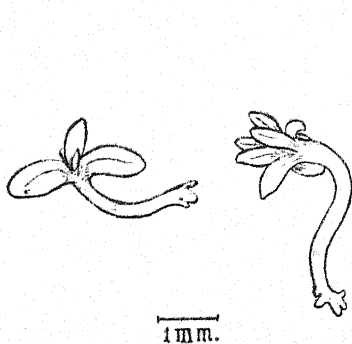


That the inability to form roots, shown by the sterile seedlings, is not due either to lack of water or to lack of aeration in the substratum, but to absence of infection by the mycorrhizal Fungus, has been fully demonstrated by subsequent cultures (p. 120).

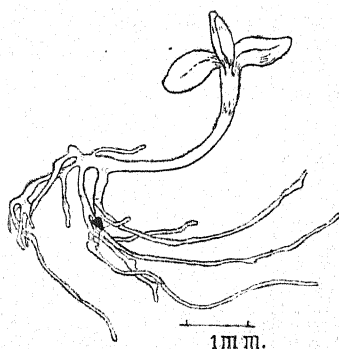
2. *Effect of organic nutrient on uninfected seedlings.* Uninfected seedlings germinated on blotting-paper or agar, and supplied with distilled water or with an appropriate solution of mineral salts, do not form roots; and show yellowing or discoloration of the leaves at an early stage.

Seedlings infected normally with the Fungus germinated under similar conditions, and supplied with distilled water only, form a well-developed root-system and several leaves (Text-fig. 3).

The failure to form roots, therefore, in the absence of infection, is not due to lack of inorganic food material, since the only differentiating



TEXT-FIG. 2. Sterile seedlings from agar cultures. Five months after sowing; four months after planting (cf. Text-fig. 3). Camera lucida drawings.



TEXT-FIG. 3. Seedling from germinator, infected normally. Five months after sowing; about four months after germination (cf. Text-fig. 2). Camera lucida drawing.

feature in the two cultures at germination consists in the presence or absence of the Fungus in the tissues of the respective seedlings.

Since also a mycelium is developed without a supply of food material from external sources, beyond the traces of impurity present, it seems clear that, at this stage and under the conditions described, the micro-organism must obtain the greater part of its food material from the seedlings, the only other source of supply being the air.

As the latter show no sign of injury, and the leaves remain green, one is tempted to suggest that the Fungus possesses, in some degree, the power of nitrogen fixation.

The remarkable vitality shown by seedlings kept for long periods on blotting-paper moistened with tap-water may possibly have some significance as indirect evidence in support of the same view.

Such seedlings form a well-developed root-system and several leaves,

which retain their green colour for several months ; e. g. a large proportion of them were alive and green at the end of *five* months. The starvation symptoms—arrest of roots, yellowing of leaves, &c., characteristic of sterile seedlings shortly after germination—are absent (Text-fig. 3).

It was thought possible, therefore, that the failure to form roots by uninfected seedlings might be correlated with some disturbance of nitrogen metabolism in the plant, assuming interchange of nitrogenous material from Fungus to plant to be a feature of the symbiosis under normal conditions.

Although without *direct* evidence to support this hypothesis, it seemed desirable to ascertain whether a supply of organic nitrogen would induce root formation, or postpone the appearance of starvation symptoms in uninfected seedlings. To test this possibility, two parallel series of sterile sand and agar cultures were carried on during several months.

The solutions used for watering the sand cultures were as follows :

Series *a*. Solution A ; 0.1 per cent. dextrose ; 0.1 per cent. Witte's peptone.

Series *β*. Solution A ; 0.1 per cent. saccharose ; 0.032 per cent. asparagin.

Series *γ*. Solution A ; 0.1 per cent. dextrose ; 0.04 per cent. uric acid.

Series *δ*. Solution A ; 0.1 per cent. saccharose ; 0.03 per cent. glyocol.

In a parallel series of agar cultures, solutions of 0.3 per cent. total concentration of Solution A were used in each case, made up with the addition of 0.12 per cent. powdered agar.

Since the result of these cultures was in every case negative, the experiments are not recorded in detail. In each series, tubes remained sterile for several months—as judged by absence of cloudiness in the media—e. g. agar tubes planted August 13, 1913, were still sterile on April 30, 1914.

The vitality of the seedlings on the different substrata varied. The only solution which appeared to be actively injurious was that used in Series *δ* to which glyocol had been added, the seedlings planted in which showed rapid discoloration of the leaves, and succumbed after a few weeks.

The greatest vitality was shown in the agar cultures of Series *a* and Series *γ*, more especially in the latter. In both cases the tubes were sterile and the seedlings alive—although moribund—at the end of six months.

The seedlings supplied with uric acid (Series *γ*) showed signs of activity after planting, but the growth initiated in the early stages was not maintained; the hypocotyl elongated, several leaves were formed, and in one case the plant produced a few small abortive rootlets. The medium in this tube

was free from bacterial or fungoid infection, and had not lost an appreciable amount of water.

All these cultures were grown in a small cool greenhouse, under the same conditions as normal seedlings in soil, special precautions being taken to prevent excessive loss of water from the tubes.

It may be inferred from these experiments that the stimulus to root development which follows entry of the Fungus into the tissues cannot be replaced by supplying the seedling with organic nitrogen in the forms mentioned.

3. *The source of infection.* Infection of the seedling root, shortly after germination, has been described in an earlier paper (3, p. 68).

The necessary observations can readily be made on seeds germinating on blotting-paper in closed dishes. Infection takes place by the outgrowth of delicate hyaline hyphae from the cells of the testa, simultaneously with — or shortly after — emergence of the seedling root.

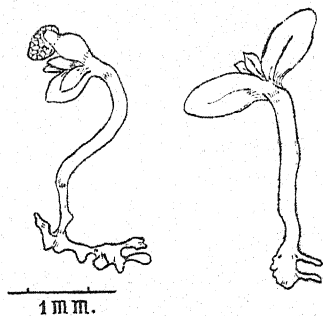
In the majority of seed cultures observed, infection has occurred with great regularity at this stage, and has been followed by the rapid development of a number of fine, transparent roots, arising adventitiously from the base of the hypocotyl. Certain irregularities have been observed nevertheless, of which at present no explanation can be given.

In a few cultures, for instance, sown in the early winter, soon after collection of the seed, infection for some reason seemed to be inhibited or delayed. These seedlings remained in a rootless condition in the seed dishes for weeks, and soon showed marked browning and discoloration of the base of the hypocotyl (Text-fig. 4); removed to another dish and placed in contact with a normally infected seedling, they at once began to develop roots.

In the paper already cited (3) it was stated that infection of the seed-coat takes place while the seeds are still in the ovary, and is independent of the bacterial and fungal infection from the air, to which all seeds are liable after they have been shed.

In order to determine the source of this infection, seeds were removed from unopened capsules for examination, and the unripe fruits containing seed investigated by means of sections cut from fixed material, embedded in paraffin or celloidin.

Thick sections of material prepared in this way make clear the manner in which infection takes place.



TEXT-FIG. 4. Seedlings from germinator; delayed infection accompanied by inhibition of root formation five months after sowing; about four months after germination. Camera lucida drawings.

The ovary of *Calluna* is four-chambered, each loculus containing a number of anatropous ovules pendent from the massive placenta at the stylar end.

When ripe, the ovary wall consists of several layers of strongly cuticularized cells, the outermost of which grows out to form a thick investment of unicellular hairs.

In some sections it is easy to observe mycelium in the fruit chambers, and about the enclosed seeds. Delicate branched hyphae are present in the cells of the wall, in the tissue of the central column, and in the funicles of the seeds. Branches from this mycelium grow across from the cells of the ovary wall to those of the seed-coat, and extend from one seed to another.

The hyphae are septate, colourless, and so transparent in fixed material as to be visible with difficulty in balsam preparations unless stained.

A microphotograph of a longitudinal section of such an unopened fruit is reproduced in Pl. VI, Fig. 1 *a*, and on this scale it is just possible to see the traversing hyphae. Parts of the same section, enlarged to show the details of infection, are shown in Fig. 1 *b*.

The degree of infection apparent in seeds removed from unopened capsules is very variable. In some, a few hyphae project from the cells of the seed-coat; in others, mycelium is difficult to find, and can only be satisfactorily seen after the seeds have been specially treated; in others, hyphae are abundant all over the seed-coat.

More especially is it difficult, as a rule, to observe hyphae on the testa of the resting seed, this difficulty of observation being due in part to the structure of the seed-coat. The latter is composed of a few cuticularized walls and an external layer of large cells which, during the final stages of development, break down, leaving at last only their 'foundations' (consisting of the inner walls and the proximal parts of the lateral walls) to form the regularly sculptured covering of the seed. The presence of the folded membranes of these cells, and of the remains of their contents, increases the difficulty of observing fine hyphae on the surface, after the seeds have become dry.

According to Church, the seeds of *Calluna* are not shed until the following spring. 'The fruits ripen in late autumn (November), and the seeds are shed in the succeeding spring, when the capsules open under desiccation' (34).

The behaviour of fruits in this respect doubtless varies with the locality and the season, but the observation does not appear to be of general application, since some difficulty has been experienced in finding unopened capsules after the middle of November.

This, although a minor point, is worth recording, because it seems not

impossible that such differences may be related with inconsistencies with regard to infection, which, as mentioned above, are sometimes observed in germinating seeds. Further, since fungal infection determines the development of the seedlings, it may also be correlated with observed irregularities in the distribution of the plant, and with the rate at which heather can become re-established on a given area by means of seed.

## THE ORIGIN OF OVARIAL INFECTION.

The question at once arises—whence come the hyphae which infect the interior of an unopened fruit?

Two possibilities evidently exist :

1. Local infection of the ovary or young fruit, from air-borne spores which reach the stigma independently, or with the pollen.
2. Infection from the root upwards, involving a distribution of the mycorrhizal Fungus throughout the plant.

The latter hypothesis has proved to be the correct one, and the facts now adduced in support of it involve the existence of a delicately balanced symbiosis of a kind not hitherto observed in any mycorrhizal plant.

The facts to be described may be summarized in advance :

1. When infection by the Fungus takes place at, or soon after germination, all parts of the seedling—root, hypocotyl, and cotyledons—are invaded by fungal hyphae.

2. In older seedlings, and in the mature plant, mycelium of the same Fungus occurs in all parts of the sub-aerial organs, in intimate relation with the tissues of the plant, and of the same nature throughout. Infection of the leaves is characteristic, and suggests a very delicate adjustment of the relations between leaf-cells and Fungus.

Infection of the ovary and other parts of the flower is, therefore, only a special case of a condition common to all the vegetative parts.

The observations on which these facts are based will be stated as briefly as is consistent with clearness, rather than in the form of a detailed cytological study.

## METHODS.

*Fixation.* A number of fixatives were tried with varying success, e. g. absolute-acetic, picric-formol, and chromo-acetic solutions of various strengths. The best results were obtained by the use of Carnoy's fluid, which has been used for the greater part of the work.

The use of a mixture of lactic acid and phenol as a clearing and mounting agent for whole roots, &c., has been found of great service, and is invaluable for the recognition of mycelium in fresh or unstained tissues.

For microtome work, material was embedded in paraffin, and the sections stained on the slide by one of the methods mentioned below. For sectioning ripe fruits enclosing seeds, material was also embedded in celloidin to avoid displacement of the seeds after removal of the paraffin.

*Staining methods.* The method found most generally useful for the differentiation of mycelium in the tissues was a concentrated solution of cotton blue (Baumwollblau 4 B) in lactic acid. Sections stained for some hours (8–24) in this solution can be differentiated in lactic acid, and examined in this reagent or in glycerine, or they may be dehydrated and mounted in balsam. By the use of whole roots, very satisfactory preparations of the Fungus in the root-cells can be obtained in this way.

The method can also be used for microtome sections if precautions are taken to prevent the sections floating off the slide.

The orceillin-aniline-blue method (Strasburger) for differentiating mycelium, and various modifications of it, were also used; satisfactory preparations of the leaves and shoot were obtained by this means, and likewise by use of iron-alum haematoxylin (Benda), and other stains in common laboratory use.

For rapid identification of mycelium in the tissues, slow maceration in sulphuric acid is useful, as is also treatment by ammoniacal cupric hydrate and chloral hydrate.

#### A. *The Seedling.*

Infection of the seedling tissues by the Fungus can be readily followed in material germinated on blotting-paper, fixed in Carnoy's fluid, stained and differentiated in 'cotton blue' in lactic acid, and mounted for examination in either pure lactic acid or a mixture of lactic acid and phenol.

Infection may begin at the tip of the root, by hyphae forcing their way between the cells of the apex; more usually it takes place simultaneously at several points, and the mycelium immediately becomes intracellular in distribution.

The hyphae penetrate cell membranes with ease; there is no trace of swelling at the point of entrance, and they ramify in the external tissues of the root as if cell-walls offered no obstruction to their growth. Infection spreads rapidly from cell to cell; some hyphal branches grow out and infect fresh rootlets as they develop; others form a tangled skein of fine hyphae in the superficial cells.

In these early stages, the mycelium is homogeneous throughout, and consists of a system of delicate colourless threads which stain deeply with 'cotton blue', both within and without the cells of the root.

The tissues of the young root, like those of healthy seedlings growing in soil, are quite colourless. In the absence of infection, arrest of growth

occurs, and this is invariably accompanied by browning and discoloration of the cell contents. Subsequent to infection, rapid elongation of the roots takes place.

In stained and cleared preparations of the young seedling, it may be further observed that infection is not confined to the rootlet, but that penetration of the tissues of the hypocotyl and cotyledons by hyphae likewise occurs and it is often possible to determine that the same mycelium is continuous from root to hypocotyl.

In the sub-aerial parts the mycelium does not develop so extensively on the surface of the plant, nor do the hyphae become massed in the superficial cells as in the roots. They are irregularly distributed in the tissues, penetrating the cell-membranes with the same apparent ease as in the root. Hyphae not infrequently grow out to the exterior, and since this may also be observed in seedlings germinated in soil, the tendency to extend outside the plant cannot be entirely due to growth in saturated air.

It is suggested that this frequent outgrowth of hyphae to the outside of the sub-aerial parts of the plant is significant in connexion with the problem of nitrogen fixation by the Fungus (p. 107). [See also Hiltner (33).]

In vertical section, the cotyledons, in contradistinction to the later leaves, show typical dorsiventral structure of the assimilating tissue: the mesophyll shows marked differentiation into palisade and spongy parenchyma; stomata are present on the lower surface, the guard-cells being slightly above the general level of the other epidermal cells.

In young seed-leaves progressive degeneration of cells of the mesophyll, such as is recorded in the mature leaf, is not apparent (p. 117), nor is there an accumulation of crystals or crystal-aggregates of calcium oxalate.

Hyphae, though not abundant, can be identified in the tissues of the cotyledon under high magnifications. They are often extremely attenuated, show a preference for the intercellular spaces, but undoubtedly invade cells of the mesophyll, some of which become filled with a tangled mass of hyphae (Pl. VI, Fig. 2).

### B. *The Mature Plant.*

Material for examination of the mature tissues was collected from many sources, and from wild and cultivated plants. In all cases the condition observed was substantially the same, and in no case—so far as my observations go—are the green parts of the plant free from mycelium.

*The Root.* The distribution and general appearance of the mycelium in the cells of the root of ericaceous plants were described many years ago by Frank for *Andromeda polifolia* (35), and more recently by Coville for *Vaccinium corymbosum* (36). My observations on the younger parts of



the mature roots of *Calluna* agree with these records, and need only be briefly described.

The outside of the young root consists of a single layer of rather large cells; the cortical tissues are much reduced in extent, and there is a slender central vascular strand. Root-hairs are absent, but a papillate outgrowth of cells may occur in the radicles of young seedlings.

The surface of the root is traversed by a network of hyaline, septate hyphae, brownish yellow in colour, and irregularly branched.

These hyphae are closely applied to the outer cell-walls and frequently grow between the cells, forcing them apart; they usually penetrate near the corners, where a cluster of branches is formed, some of which pierce the cell-wall. Within the cells they develop several stout coils, from which, at intervals, crowded clusters of short thick branchlets are given off, completely filling the cell (Pl. VI, Fig. 11). Almost every cell of the younger part of the root is infected in this way, and in surface view such cells present an appearance as if filled with dense coils of closely interwoven hyphae.

In addition to this characteristic infection, a system of finer hyphae is present, the branches of which are often especially abundant around the root-tip, penetrating cells in the same way, and forming coiled masses within them.

With the exception of the characteristic 'clusters' in root-cells described above, the mycelium *within* the plant tissues is distinguished from the same mycelium outside only by the smaller diameter of the hyphae composing it, the two systems of hyphae being continuous. Outside the cells, the mycelium sometimes exhibits the structure shown in Pl. VI, Fig. 3. The hyphae figured are very transparent, of rather large diameter, and show characteristic swellings at intervals. This photograph may be compared with Pl. VI, Fig. 4, which shows hyphae, identical in structure, from a pure culture of the Fungus extracted from the ovary, growing on *Calluna*-extract gelatine (p. 129).

These swellings have not been observed on the hyphae of the inter- or intracellular mycelium. They do not seem to correspond to the 'vesicles' of Gallaud and other observers, but are apparently formed by the vegetative hyphae when growing saprophytically, either in proximity to the root, or on certain artificial media.

*The Shoot. Historical.* The members of Ericaceae possess anatomical peculiarities—especially with regard to leaf structure—which have led to repeated investigation by plant anatomists.

In particular, the ericoid leaf has attracted attention as a characteristic xerophytic type, and the variations of structure shown by the leaves of the several members of the group have been recorded in great detail, and used as a basis for classification.

A summary of the chief features of general anatomical interest in various genera has been given by Solereder (37).

The details of leaf structure in the members of the sub-order Ericoideae have been the subject of a monograph by Ljungström (38), who used his observations as a basis for subdivision of the group into four classes, each distinguished by characteristic leaf structure; in the fourth of these classes he places *Calluna* together with *Erica dianthifolia*.

The Vaccinioideae, Arbutoideae, and Rhododendroideae have also been monographed from this point of view by other observers (39, 40).

Contributions to the comparative anatomy of the members of the order have been made also by Simon (41).

These observers have drawn attention to various features of interest in the anatomy of the leaf: the characteristic shape, with corresponding anomalies in the distribution of the assimilating tissues; the complicated structure of the cell-walls and cuticle; the distribution and structure of many different types of covering and secretory hairs; and the presence of accumulations of calcium oxalate in the tissues of the stem and leaf, either as single crystals or crystal-aggregates.

There is no record in these papers of any observations dealing with a regular infection of the tissues of the shoot by fungal hyphae, such as is about to be described.

In view of the very full accounts given in the papers cited above, a brief summary of the anatomy of the leaf will suffice.

The various species of *Erica* and its allies have often been described as possessing 'rolled leaves', a misleading title since the characteristic hollowing of the under side does not arise by a *rolling back* of the upper side of the leaf, but is developed secondarily as a groove or furrow (or several such) on the lower side.

The leaves of *Calluna* are decussate in arrangement, of small size, and of the familiar ericoid type. The mature leaf is almost quadrangular in section, the sides tapering gradually to the abaxial surface, of which most of the width is occupied by a single conspicuous groove, the walls of which are beset with hairs.

The adaxial side of the leaf is adpressed to the stem, and owing to this, and to the shape of the leaf, the flanks receive most illumination; correlated with which the bulk of the chlorophyllous tissue is found beneath the epidermis in this region.

Stomata are present on both sides of the leaf.

The assimilating tissue in the mature leaf has lost all trace of the dorsiventrality found in the cotyledon, and the arrangement of the assimilating tissue—as in other ericoid types—is closely correlated with the shape and position of the leaf, and its consequent illumination.

Chlorophyllous tissue is practically absent from the upper side of the

leaf; the upper epidermis roofs over an almost continuous air-space, traversed only by the branches of the central vascular strand and the tissues which surround it (Pl. VI, Fig. 5).

In median longitudinal sections parallel to the upper and lower surfaces, the relations of the large air-spaces to the vascular strands and to the assimilating tissue below the epidermis of the lateral face of the leaf are made clear. A few layers of assimilating cells on either side pass internally into spongy mesophyll, consisting of strands of green cells, which bridge over at intervals the space between the assimilating tissue and the parenchyma surrounding the bundles.

Towards the base of the young leaf there is a continuous tissue of thin-walled cells. In the mature leaf this region is filled with the remains of these cells intermingled with a dense accumulation of crystal-aggregates of calcium oxalate.

All stages of degeneration are to be observed in these cells and in those forming the trabeculae. Empty cells may be seen, each filled by a single large crystal-aggregate, or groups of such crystals lie free in the space, entangled among the walls of the cells which originally contained them. The cells are represented often only by a framework of walls, though sometimes traces of the cell-contents are recognizable (Pl. VI, Fig. 6).

The presence and distribution of the fungal hyphae in these tissues will now be described.

#### THE DISTRIBUTION OF THE FUNGUS.

Longitudinal sections through the young shoots provide abundant evidence of the prevalence, on the outside of the stem and leaves, of a network of fine hyphae, ramifying among the hairs or closely applied to the cuticle of the epidermal walls. These hyphae are often excessively fine and are easily overlooked in unstained preparations.

In fairly thick hand-sections or in serial sections of the shoot, it may be determined that this external mycelium is part of a system which pervades the tissues of the stem and leaves. Hyphae penetrating the epidermal cells can be traced into the deeper-lying tissues of the leaf and into close contact with cells of the mesophyll.

The microphotograph produced in Pl. VI, Fig. 7 shows a tangle of hyphae between the leaves of a young shoot, branches from which penetrate the tissues of the leaves on either side.

The hyphae show no preference for special points of entrance or egress. They penetrate with equal ease the cuticularized cells of the epidermis or the base of a hair, and may be occasionally seen emerging from the large hair which terminates the leaf apex.

In sections which have been specially treated and stained, it is possible to follow the ramifications of these fine hyphae across the air-spaces and into the cells of the mesophyll. They are often of extreme tenuity,

and in the absence of the mass of cumulative evidence available doubt might arise as to their true nature.

The staining reactions of the hyphae in contact with the mesophyll cells become affected and the appearance of many of these cells is consistent with an active disintegration and digestion of the invading mycelium.

The difficulty of finding active mycelium in the leaf seems to be due chiefly to the fact that in the unaltered condition it is present in the intercellular spaces only, these being relatively of such large extent that the contained hyphae are usually torn out or displaced in sections.

It is possible, however, to obtain preparations which provide conclusive evidence of the presence of functional hyphae in the leaf tissues and of their invasion of mesophyll cells.

In the chlorophyllous cells, progressive stages of degeneration may be observed; fragments of mycelium, irregular in outline but still stainable, can be found in close contact with, and penetrating such cells; many of them obviously contain hyphae in various stages of degeneration, while the same process of disintegration is affecting the cells themselves.

Plasmolysis in various degrees, alteration of staining properties, degeneration of the chloroplasts, and finally of the whole contents of the cell, mark progressive stages in the process. By suitable staining methods, strands of very attenuated mycelium can be differentiated, bridging across the spaces, sending branches to the cells, and demonstrably continuous in places with those on the outside of the leaf (Pl. VI, Fig. 12).

Calcium oxalate is present in remarkable abundance in the leaves in the form of large crystal-aggregates.

The crystals accumulate chiefly in the mesophyll tissue towards the base of the leaf, and are conspicuous macroscopically in small pieces of shoot, cleared in cedar-wood oil.

In leaf sections, the crystals are most abundant in the cells of the spongy mesophyll in the lower half of the leaf; single aggregates are present in the empty cells of the trabecular tissue, completely filling them, and occur also in groups intermingled with the cells and cell-remains in the central part of the basal region of the leaf (Pl. VI, Fig. 6).

Hyphae may be recognized in close contact with the crystals; sometimes free in the intercellular spaces; more often in the form of strands or fragments of greenish membrane mingled with the remains of the cell-walls (Pl. VI, Fig. 13).

A network of fine mycelium with looped masses of hyphae may often be found in the cells of this part of the mesophyll, and can be identified after treatment of the section with concentrated sulphuric acid.

There is no doubt that the region of the leaf which contains most abundant evidence of the presence of mycelium is also the region in which the greatest accumulation of calcium oxalate occurs.

Whether this association of hyphae and crystals is accidental, or whether it is one of the links in a chain of events which connects the metabolism of the plant with that of the Fungus, remains an open question; but the presence of such relatively enormous accumulations of a calcium salt in the tissues of a plant believed to be unusually intolerant of lime salts in the soil raises many points of interest.

The oxalic acid may be produced by the plant cells or by the Fungus; if by the former, it may be an indication of interference with normal cell metabolism, induced possibly by the increased demand upon the available oxygen supply.

If the calcium absorbed by the plant from the soil, in the form of calcium salts, is constantly in requisition for the neutralization of oxalic acid, the edaphic preferences of the plant for acid soils and those deficient in lime salts are puzzling.

If, on the other hand, the calcium salt is derived immediately from the Fungus, it must be obtained either from the soil (which raises the same difficulty), or from some part of the plant tissues, e. g. the middle lamella of the cell-walls.

The establishment of the fact that the relation existing between the plant and the Fungus is an obligate one permits the problem of the origin of the calcifuge habit to be stated in a new form, and hence provides a fresh point of departure for experimental research into this important phenomenon.

Assuming that the Fungus forms and excretes oxalic acid—and possibly makes its primary attack upon the plant by this means—it may well be that the amount of calcium salts present in the plant at the moment of infection is a decisive factor in determining whether infection shall or shall not occur.

If the amount is considerable, the plant will resist the attack of the Fungus, and, by its very resistance, cuts off all chance of growth on normal calcareous soils, the presence of the Fungus having become one of the first essentials to development on the part of the seedling.

If, on the other hand, the plant cells are deficient in lime salts, the excess of oxalic acid can act, directly or indirectly, by preparing the way for the entrance of the parasite, symbiosis is established, and, so long as equilibrium is maintained, the plant flourishes.

This working model of the conditions underlying the origin of the 'lime-shy' habit makes no claim to completeness, but, as already stated, it immediately suggests fresh methods by which to attack the problem experimentally.

The excretion of calcium oxalate by Fungi growing in plant tissues or on artificial media is not uncommon (42), and an alteration of the calcium oxalate content of the cells of the host has recently been noted as one of

the indirect results of invasion of leaves by parasitic Fungi (43, 44). The discussion of hypotheses to account for the storage of a calcium salt in the plant tissues may profitably be postponed until more data are available as to the nutritive preferences of the Fungus.

*The Stem.* The presence of mycelium on the outside of the young stem and the penetration of branches from it into the underlying tissues have already been noted (p. 116). Evidence of the presence of mycelium may be found in most tissues of the stem, e. g. in the parenchyma of the cortex, and in that associated with the vascular tissues; the cells of the pith, also, probably contain hyphae in a vestigial condition.

The tissues of the pith and of the cortex likewise contain quantities of calcium oxalate in the form of large crystals and crystal-aggregates. Untreated sections are often rendered absolutely opaque by the presence in every cell of these crystals. In rare cases small fragments of hyphae which stain deeply with cotton blue or aniline blue can be identified in the neighbourhood of crystals; more frequently the hyphae are present in an extremely attenuated condition, are very transparent, refuse to take up the characteristic stains, and can only be identified with difficulty.

The most satisfactory method of obtaining conclusive proof of the presence of mycelium in such tissues is by slow maceration in sulphuric acid, or by prolonged treatment with clearing agents, such as chloral hydrate, eau de Javelle, or potash, before staining.

#### 4. *The Isolation of the Fungus.*

During the past three years repeated and fruitless attempts have been made to isolate the mycorrhizal Fungus from the root-cells.

The method first adopted was to remove clean transparent young roots from the outside of a ball of soil surrounding the roots of a pot plant. These roots were washed in fast-running water for twenty-four hours, rinsed repeatedly in sterilized distilled water, after which small pieces were planted out on agar plates. (Direct sterilization of the surface of the root, by heat or momentary immersion in weak solutions of mercuric chloride, was found to be impracticable.)

Using this method, a number of fungal species were isolated, none of which, however, satisfied the test of successful inoculation into sterile seedlings.

Among those constantly found were species of *Cladosporium*, *Penicillium*, *Citromyces*, *Fusarium*, and *Alternaria*, all of which are apparently constant or fairly constant members of the epiphytic microflora of the roots. A species of *Cladosporium* was invariably present in my cultures, and is always dominant if unsterilized seedlings are planted in agar.

The hanging-drop method of culture was tried with the same lack of success. Pieces of washed root were transferred to hanging drops of sterile water, and to similar drops of various solutions, which it was

hoped might favour the growth of the endophyte, as compared with that of its more epiphytic competitors; neutral and slightly acid sugar solutions, soil extracts, peat extract, *Calluna* extract, were all used for this purpose.

In many of these drop cultures the endophyte was obviously active, but on transferring to plate cultures, the growth, if present, was always masked by that of one or several of the Fungus species mentioned above.

Attempts were also made, using similar hanging-drop cultures, to extract the Fungus from the seed-coat of the resting seed, and from seedlings soon after infection.

In several cases interesting results were obtained, throwing light on the formation of bacterial colonies on the roots of seedlings, as described in an earlier paper (3), but the results were negative in so far as the isolation of the endophyte was concerned.

The discovery of mycelium within the ovary, and of its distribution throughout the plant, provided a fresh starting-point. Unripe capsules were sterilized by passing them through a flame, or by immersion in 1 per cent. mercuric chloride. The seeds and internal tissues were then removed and transferred with aseptic precautions to agar plates.

Colonies of a non-sporing Fungus developed on several of these plates, both from seeds and from pieces of ovary tissue, and were subcultured on various media. The Fungus isolated in this way was used for the inoculation of sterile seedlings. These seedlings, immediately after infection, developed a root-system and grew vigorously in a perfectly normal manner under aseptic conditions in closed tubes (Pl. VI, Fig. 14).

The agar medium in which they were planted was similar to that used previously, without success, for the cultivation of sterile seedlings (p. 106).

As in former cultures, uninfected controls remained rootless, and in other ways showed more or less complete inhibition of growth (Pl. VI, Fig. 15, *a* and *b*).

Demonstration of an obligate relation between the plant and the Fungus which besets its roots, and of the identity of this Fungus with that isolated from the ovary, is therefore complete.

*Uninfected* seedlings, grown under strictly aseptic conditions, or subjected to casual infection by Fungi and Bacteria from the air, fail to form roots.

Similar seedlings, infected with a Fungus isolated from the ovary of the flower morphologically identical with that in the cells of the root, develop a root-system, and continue to grow normally under aseptic conditions, the rooting medium being alike in the two cases (cf. Pl. VI, Figs. 14 and 15).

Only as an embryo in the resting seed does the heather plant retain an



independent existence; from the moment of germination onwards it is a dual organism, the artificial synthesis of which has now been accomplished. The endosperm and embryo are the last strongholds of independence retained by the plant.

It is interesting to speculate on the course of the evolutionary path which has been traversed, and to inquire if this condition of dependence has been reached as the result of a long series of capitulations on the part of the plant, each marking an extension of the area in which the Fungus is suffered as an endophyte in the tissues.

When first extracted, the Fungus makes a rather weak growth on artificial media, but becomes more vigorous when subcultured.

These differences in vigour are correlated with corresponding differences in the ease with which seedlings can be successfully inoculated.

Thus, seedlings inoculated from a sub-culture growing on *Calluna*-extract gelatine immediately formed roots and continued to grow vigorously (Pl. VI, Fig. 14), while seedlings of the same age, inoculated from a strongly-growing old culture on rice, formed roots, but were at once partly or completely parasitized by the Fungus which became conspicuous externally on the leaves, or, in the case of weak seedlings, killed them outright before a root-system was formed (Pl. VI, Fig. 16).

In the former case the endophyte grew almost entirely below the surface of the rooting medium; in the latter it developed vigorously on the surface.

Further investigations are in progress with regard to these differences of behaviour, which are evidently closely correlated with the nutritive conditions before and after infection.

This was strikingly apparent in the two cultures from which representative seedlings are figured on Pl. VI, Figs. 17, 18, and of which the history was as follows. Sterile seedlings were planted out on filter-paper in sterile tubes, inoculated from the vigorous rice culture mentioned above and supplied with distilled water, with the result that roots were formed in every case, although some of the seedlings showed signs of being too strongly invaded (Pl. VI, Fig. 17).

Seedlings similarly inoculated and planted out on paper, but supplied with a nutrient solution (Solution A (p. 106), 0.15 per cent. total concentration) instead of distilled water, were immediately attacked by the Fungus and destroyed, the mycelium subsequently forming an extremely vigorous growth on the paper (Pl. VI, Fig. 18).

The results of these isolation and inoculation experiments may be summarized as follows:

1. A Fungus species, showing identical morphological characters in each case, has been isolated from the unopened fruit and from seeds removed from the unopened fruits of *Calluna vulgaris*.

2. Sterile seedlings, inoculated with this Fungus (from either source), subsequently produce normal root and shoot systems when grown in closed tubes, under aseptic conditions.

3. Control seedlings remain rootless.

4. The ease with which plant and Fungus can be successfully synthesized depends upon the nutritive and other conditions under which the experiment is conducted, the age of the seedling, the age and vigour of the fungal culture used, and, in all probability, upon the composition of the nutrient material upon which the latter was cultivated outside the plant.

5. The Fungus isolated in this way is morphologically identical with that present in the root mycorrhiza.

#### THE ENDOPHYTE.

*Inside the plant.* The vegetative mycelium as it appears in the various organs of the plant has already been fully described (p. 112). With the exception of the characteristic clusters of branches which fill the root-cells (Pl. VI, Fig. 11), and the swellings on hyphae outside the root but continuous with those in the tissues (Pl. VI, Fig. 3), no special organs have been observed. There is no doubt but that the distribution of the endophyte in the artificially synthesized plant is as wide as that described for normal seedlings; there is no reason to believe that the details of development in the tissues differ in any respect from those described for the latter.

*The formation of tubercles.* Invasion of the roots of a vascular plant by either Fungi or Bacteria is often marked by the formation of tubercles or nodules. The root tubercles of Leguminosae and the nodules on the roots of *Alnus glutinosa* and *Podocarpus* are familiar examples.

So far as I am aware, there is no record of tubercles on the roots of an ericaceous plant, and their formation must, therefore, be relatively rare.

Tubercles of a quite characteristic kind are, however, formed sometimes by healthy plants of *Calluna* growing on typical heath soils.

The tissues of these tubercles contain fungal hyphae and Bacteria. The details of their structure and the relations, if any, between the two micro-organisms is being investigated and may throw light on the nature of the bacterial colonies present on the roots when growing in certain soils.

*Outside the plant.* Sub-cultures of the Fungus from the original colonies derived from the plant tissues were made on rice and on gelatine-*Calluna* extract.

The behaviour of the organism on these and other media, together with a detailed account of the morphological characters, will be found in the Appendix (p. 128).

Unless otherwise stated, all the cultures described were made from this original sub-culture on rice, and were kept at room temperature.

The morphological characters of the Fungus agree with those of the genus *Phoma*.

They coincide generally with those described for the five pycnidia-forming Fungi, isolated from the roots of five ericaceous species by a previous observer (26), and referred to this genus.<sup>1</sup> In view of the anomalous distribution in the tissues of the plant, and the fact that the members of the genus *Phoma*, as commonly understood, are obligate parasites in all parts of plants *except the leaves*, it is suggested that the species now described should be placed in a new sub-genus *Phyllophoma*.

With regard to the physiology of the Fungus, many problems present themselves.

The reaction of the micro-organism to acid and alkaline media and to various soil extracts; the possible excretion of acid by the Fungus when grown on various substrata; a more detailed knowledge of the nature of the enzymes produced; and the behaviour of the Fungus in pure culture when inoculated with Bacteria present on the roots of plants growing in calcareous soil, all suggest lines of research of special interest from the point of view of the ecology of the plant.

#### THE DISTRIBUTION OF THE ENDOPHYTE IN OTHER SPECIES OF ERICACEAE.

A number of other ericaceous species have been investigated in order to determine whether the condition described for *Calluna* is common to other members of the order.

In every species examined, mycelium could be identified in the ovary of the unopened flower—in many cases removed from the resting bud for examination—which showed relations with the plant tissues similar to those described for *Calluna*. It has not yet been possible to make a detailed examination of the vegetative tissues in each case, but in some of the species the endophyte is undoubtedly present in the leaves also.

The ease with which mycelium can be demonstrated varies very much. In the members of Ericoideae it is usually extremely reduced and difficult to recognize in the tissues.

Since the list of species appended contains representatives from the Rhododendroideae, Arbutoideae, and Vaccinioideae, as well as from the Ericoideae, it seems reasonable to conclude that the distribution of the endophyte described in detail for *Calluna* is common to all members of Ericaceae. The agreement of members of Vaccinioideae in this respect is of interest

<sup>1</sup> It is doubtful whether any of these species can be identified with certainty with the species of *Phoma* recorded by Rabenhorst from members of Ericaceae (see Ternetz, 26).

for systematic reasons, and favours the view that the members of this sub-order are closely related to the hypogynous forms.

Since ovarial infection is the rule, the seeds of all species will evidently always be liable to infection by the specific Fungus; whether the relation between seedling and Fungus is of the same obligate character as has been demonstrated for *Calluna* can only be determined by experimental investigation of each case. The observation recorded by an earlier investigator, that young seedlings of *Andromeda polifolia* germinating viviparously in the capsule showed fungal infection of the roots, is worthy of notice in this connexion (26).

Experimental investigation is also required in order to determine whether the endophyte is specific to each plant species, or can be used successfully for the inoculation of any member of the group in which an obligate relation can be demonstrated.

Appended is a list of the species in which ovarial infection has been observed, and a similar distribution of the Fungus may probably be inferred.

#### RHODODENDROIDEAE.

*Ledum palustre*, *Rhododendron ponticum* (Garden var.), *Rhododendron indicum* (*Azalea indica*, garden var.), *Rhododendron sinense* (*Azalea sinense*, garden var.), *Leiophyllum buxifolium*, *Kalmia angustifolia*.

#### ARBUTOIDEAE.

*Pieris floribunda*, *Pieris japonica*, *Gaultheria acutifolia*, *Arctostaphylos Uva-ursi*, *Arbutus Unedo*.

#### VACCINIOIDEAE.

*Vaccinium Vitis-idaea*, *Pentapterygium serpens*.

#### ERICOIDEAE.

*Calluna vulgaris*, *Erica carnea*.

#### SUMMARY.

1. In common with other members of Ericaceae, *Calluna vulgaris* possesses a characteristic root mycorrhiza.
2. Infection by the mycorrhizal Fungus takes place shortly after germination, the source of such infection being the testa of the seed.
3. Infection does not cease with the formation of the characteristic mycorrhiza associated with the roots but affects all parts of the young seedling.
4. In the mature plant, likewise, the Fungus is not confined to the roots or colourless parts, but is present also in the sub-aerial organs—in the tissues of the stem, leaf, flower, and fruit.

5. The ovary—and later the young fruit—contains mycelium in all parts of the internal tissues. This mycelium infects the seed-coats of the developing seeds.

6. The embryo and endosperm of the resting seed are free from infection.

7. By appropriate methods, seeds can be sterilized and seedlings germinated, free from fungal and bacterial infection.

8. Failing infection by the appropriate Fungus, such seedlings do not develop roots; they suffer complete inhibition of growth, remaining alive, but rootless, for several months.

9. The mycorrhizal Fungus has been isolated from unopened fruits and from seeds removed from unopened fruits and has been grown in pure culture; sterile seedlings inoculated from a pure culture of this Fungus develop normally under aseptic conditions. The synthesis of Fungus and plant has thus been accomplished.

10. In morphological characters the Fungus resembles the genus *Phoma*. In view of its distribution in the plant, and the unusual biological relations exhibited, it is proposed that the species described must be placed in a new sub-genus, for which the name *Phyllophoma* is suggested.

11. Ovarial infection has been observed (and a similar distribution of the Fungus in the vegetative parts inferred) for a number of ericaceous species, including members of the *Vaccinioideae*.

12. It has not been found possible to replace the stimulus to development which follows seedling infection by supplies of various organic nitrogenous substances in the food material.

#### DISCUSSION OF RESULTS.

In view of the facts described in this paper, the conclusions of Stahl, with regard to the relations between ericaceous plants and their mycorrhizal Fungi, require revision.

As a result of experimental work on *Vaccinium*, &c., germinated and grown in heath soil, sterilized by heat and by ether vapour, Stahl summarizes his conclusions as follows:

‘Während manche obligaten Mycorrhizenpflanzen, wie wir früher gesehen haben, der Anzucht aus Samen und der Kultur grosse Schwierigkeiten bereiten, lassen sich die Ericaceen auch ohne Gegenwart von Wurzelpilzen unschwer kultiviren, und ihre Samen gehen, zwar oft langsam, aber in grossem Procentsatz und sicher ohne Mitwirkung symbiotischer Pilze auf’ (16).

According to Stahl, seedlings of *Vaccinium Myrtillus* sown in May, on soil which had been previously heated or treated with ether vapour, when examined in October of the same year were completely free from fungal infection (‘völlig pilzfrei’).

As a general statement affecting all members of the Natural Order Ericaceae, the conclusions expressed in the passage quoted above can no longer be accepted.

It is true that an obligate relationship with the mycorrhizal Fungus cannot, at present, be predicted with certainty for every member of the group, but, in view of the fact that some species of *Vaccinium* (p. 124) show ovarial infection of the kind described for *Calluna*, a repetition of Stahl's experiments, under conditions in which negative evidence regarding infection of the roots can be more satisfactorily tested than by microscopic examination only, is required.

Recent work by Russell (45), on the sterilization of soil by heat and antiseptics, appears also to have a bearing on the interpretation of the results in these and similar experiments carried out by Stahl.

The condition described for *Calluna* does not seem to have an exact counterpart among mycorrhizal plants.

The dependence of the plant on the fungal symbiont is paralleled, if not exceeded, among the Orchids, in some species of which development of the embryo ceases at an early stage, unless infection occurs. In the Orchids, however, the endophyte is strictly confined to the non-chlorophyllous tissues, and this restricted distribution is not accidental, for Bernard has shown that portions of the stem of some Orchids have a poisonous effect upon the mycorrhizal Fungus of the same plant. In cultures, the fluid diffusing from these tissues killed the hyphae; heated to 55° C., the toxic properties disappeared, from which it was inferred that the poisonous substance was probably of the nature of an enzyme (46).

While also exhibiting complete dependence upon the Fungus at an early stage in the life-history, *Calluna* has an advantage over the Orchids, since the seeds are insured against the risk of non-infection—no small advantage in the case of small, light seeds, distributed by wind. This advantage would seem to be counterbalanced by the presence of mycelium of a facultatively parasitic nature in the tissues of the shoot.

There is no evidence for nitrogen fixation from the air by the Orchid Fungi, but it is usually believed that the Orchid plant obtains nitrogenous food material from the Fungus by the digestion of mycelium in specialized cells ('Verdauungszellen').

It is now suggested that *Calluna* and its allies have solved the nitrogen problem in a different way.

The toleration by the plant of mycelium in the intercellular spaces of the leaves continuous with that on the outside of the shoot, and the ultimate relations of the hyphae with the mesophyll cells, point to the possibility of the fixation of atmospheric nitrogen in some degree by the Fungus.

Indirect evidence favouring the same view has already been cited

(p. 107), while nitrogen fixation has been claimed for five pycnidia-bearing Fungi isolated from members of Ericaceae, growing in pure culture outside the plant (26). The possibility of growing the artificially infected plant of *Calluna* in a nitrogen-free substratum awaits experimental proof.

The only green plant for which has been described a like distribution of mycelium—not obviously pathogenic—in the tissues, combined with ovarial infection of the seeds, is the Darnel grass (*Lolium temulentum*).

In this case, however, the plant does not form mycorrhiza, nor has it been established that the relation with the Fungus is an obligate one (47, 48).

Some degree of symbiosis has been inferred, but the experiments of Hiltner (33) to establish nitrogen fixation for this Fungus are inconclusive.

The case of the saprophytic Orchid *Gastrodia elata*, described recently by Kusano (49), is of interest, since there is dependence of the plant upon fungal infection for a part of the life-history only, coupled with a strictly limited distribution of the Fungus in the tissues.

The higher symbiont in this curious relationship is a rootless saprophytic species of Orchid; the fungal partner is *Armillaria mellea* ('*Rhizomorpha subterranea*'), a well-known facultative parasite. The association is an obligate one for the plant, in so far as the flowering stage is not reached unless 'mycorrhiza' is formed, but the vegetative period of the life-history is independent of infection, which takes place only occasionally in nature. It involves symbiosis, by means of a stem mycorrhiza, on the part of two heterotrophic plants, the fungal partner remaining apparently unmodified by its temporary association with the plant.

The view is put forward by this author, that the first step towards the formation of mycorrhiza can here be recognized, involving a temporary modification of parasitic habit on the part of the Fungus concerned.

It is evident, from a consideration of mycorrhiza in general, that it is still impossible to frame a definition which will include all the known cases. The theories of earlier observers implied a strict symbiosis with reciprocal advantages of an obvious kind. Later workers tend rather to regard the relation as primarily one of *parasitism* on the part of the Fungus, tolerated and often turned to account by the plant, or even become indispensable to it.

Thus Gallaud (20) concludes that there is no 'symbiose harmonique' between plant and endophyte. The latter is only an internal saprophyte of a special kind which the plant cells can keep in check without preventing further development.

Bernard holds similar views with regard to the Orchids:

'A un point de vue théorique il résulte de ces constatations que l'état dit de symbiose est en quelque sorte un état de maladie grave et prolongée, intermédiaire entre l'état des plantes atteintes d'une maladie rapidement



mortelle et celui des plantes qui jouissent d'une immunité complète' (loc. cit.).

A similar interpretation may perhaps be suggested by the condition in Ericaceae, many of the members of which have solved the problem of growth upon the poorest and most unpromising soils, but have solved it at the price of their independence.

A study of these delicately-balanced relations inevitably provokes comparison with phagocytosis and the phenomena of immunity as observed in animals.

The recent work of Fellmer (50), and of Wendelstadt and Fellmer (51), is suggestive in this connexion as indicating how the *specificity* shown in relations between a vascular plant and a Fungus, such as those described above, or possibly in symbiotic associations in general, can be brought into line with the facts of immunity and reaction to immune sera in animals.

These authors have shown (1) that plant extracts ('Eiweissstoff'), when injected into animals, produce *specific* precipitin reactions, with anaphylactic phenomena quite analogous to those produced by the injection of animal sera; and (2) that similar specific reactions are given by animals to extracts of Fungus protoplasm prepared in the same way.

The attempt to use the latter to produce immunity in the higher plants against attacks of Fungi, although theoretically possible, has not yet proved successful in practice.

Only investigation of special cases can demonstrate with certainty the exact relation between the degree of development of the Fungus in the ericaceous plant and the well-being of the latter for any given conditions of growth, or provide a key to the soil preferences of these plants, both in the field and under cultivation.

It is unlikely that observed peculiarities in this respect can always be explained as consequences of the xerophytic habit, with the decreased transpiration current and retarded absorption which this entails.

I am indebted to Dr. R. Stenhouse Williams for testing the sterility of seedlings used in inoculation cultures, and to Professor Keeble for helpful criticism of this paper.

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## APPENDIX.

### 5. *The Endophyte in pure Culture.*

#### 1. *Rice and tap-water.*

*Thirty days' culture.* Vigorous superficial growth; mycelium snow-white at first, becoming greyish brown. The medium slowly changes colour from yellow to dark brown.

*Six weeks' culture* (Pl. VI, Fig. 19). As last, but many small dark dots on the

bared parts of the surface, indicating the formation of pycnidia. Older mycelium greyish brown; younger mycelium from germination of the pycnidiospores snow-white. Mycelium resembling that found in the root mycorrhiza of the plant. Larger hyphae hyaline, regularly septate, yellow-brown, diameter 0.007–0.008 mm., giving rise directly or by gradual transition to fine, colourless, branched hyphae; the ultimate branches very attenuated.

Larger hyphae rarely shortly jointed in growth or swelling to form terminal or intercalary swellings; often forming strands of parallel-growing hyphae. The colourless vegetative hyphae become densely interwoven, but there is no formation of a definite stroma.

Pycnidia produced freely on the surface; globose or slightly irregular in shape, with apical papilla; size variable, diameter from 0.2 mm. upwards; colour dark brown (Pl. VI, Fig. 8).

Pycnidiospores oval, 0.003 mm.–0.004 × 0.002–0.003 mm.; wall pale greenish yellow to yellow-brown (Pl. VI, Fig. 9).

## 2. *Calluna-Extract Gelatine.*<sup>1</sup>

*Ten days' culture.* Colony 4 cm. diameter. Superficial growth of mycelium, well developed and snow-white in colour.

*Fifteen days' culture* (Pl. VI, Fig. 20). Colony 6.5 cm. diameter. Mycelium snow-white. Gelatine slowly liquefying.

Mycelium vigorous; hyphae branched, septate, colourless to yellow-brown, resembling those from culture on rice, but in older cultures the majority form characteristic swellings—intercalary, terminal, or in chains. Mycelium often forming parallel strands.

No formation of spores or pycnidia observed.

Complete liquefaction of the gelatine in older cultures.

## 3. *Water-culture solution A* (p. 106) and 0.12 % Agar.

*Twelve weeks' culture.* Medium slightly clouded, colour unchanged. Growth on surface feeble or absent, except for a slight growth of hyphae on the sides of the tube. Mycelium homogeneous, composed of fine, branched, colourless hyphae, frequently anastomosing. The mycelium in this culture was identical with that observed in the early stages of infection of sterile seedlings.

## 4. *Solution A + 0.1 % Dextrose + 0.1 % Witté's Peptone + 0.12 % Agar.*

Twenty days' culture. Colony 7 cm. diameter.

Thirty „ „ Colony covered plate.

Growth colourless; scanty white mycelium on surface.

In older cultures the medium darkens very slowly, becoming yellow to yellowish brown.

Growth vigorous, chiefly below surface. Hyphae colourless to yellow-brown in old cultures. Older hyphae often closely septate and with thicker walls; no separation of chlamydospores observed. No formation of swellings in cultures up to five months old. Refractive droplets abundant in hyphae and as an excretion in the medium. No formation of spores or pycnidia observed.

<sup>1</sup> 130 grm. of young shoots of *Calluna* extracted in water; extract made up to 1 litre, and 120 grm. sheet gelatine added = dark-brown medium.

5. *Nutrient Agar*,<sup>1</sup> + 10 acidity.

Twenty days' culture. Colony 3 cm. diameter.

Thirty " " Colony 3.25 cm. diameter.

Forty " " Colony 3.5 cm. diameter.

Colony yellowish, raised, with very scanty growth of mycelium on surface. Colour of the medium unchanged. Mycelium showing a preponderance of the larger hyphae, in which the cross-walls are strongly marked; branches often 'jointed' in structure; no separation of chlamydospores observed (Pl. VI, Fig. 21).

The mycelium shows a vigorous development of large swellings—terminal, intercalary, and in chains.

6. *Nutrient Agar*, -5 alkalinity.

Twenty days' culture. Colony 1.5 cm. diameter.

Thirty " " Colony 1.8 cm. diameter.

Forty " " Colony 2.0 cm. diameter.

No further growth. Colony similar in appearance to last, but the medium tending to deepen in colour.

Mycelium as last, with very conspicuous development of swellings.

*Hydrolysis of arbutin.* The glucoside arbutin is widely distributed among the members of Ericaceae.

It occurs abundantly in the tissues of *Calluna*; in a watery extract of the leaves and young shoots made in the cold, the colour, at first yellowish, slowly deepens until the solution becomes dark brown.

This browning of the extract is presumably due to hydrolysis of the glucoside to glucose and hydroquinone, the latter of which oxidizes to form a brown pigment.

The browning of the plant-cells in unhealthy seedlings is possibly due to related causes.

The production of an enzyme by the Fungus able to effect the hydrolysis of arbutin has been determined by transferring mycelium from a pure culture on rice to tubes containing a 0.5 % solution of arbutin. At the end of a week the tubes showed slight browning of the solution, the coloration gradually becoming more marked. Control tubes, containing a 0.5 % solution of arbutin without the Fungus, remain colourless.

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<sup>1</sup> The nutrient agar was made up with Lemco and standardized in the usual way.

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## EXPLANATION OF PLATE VI.

Illustrating M. C. Rayner's paper on Obligate Symbiosis in *Calluna vulgaris*.

Figs. 10, 15 b, 16, 17, 18, 19, 20 = photographs. Fig. 21 = microphotograph. Figs. 11, 12, 13 = camera lucida drawings. Figs. 14, 15 a = drawings.

Fig. 1 a. Ovarial infection. Part of a longitudinal section of the unopened fruit, showing seeds still attached to funicles. Mycelium in all parts of the ovary tissues, and traversing the spaces between ovary wall and seeds, &c. *h.* = hyphae.  $\times 60$  diam.

Fig. 1 b. Part of the section shown in Fig. 1 a, more highly magnified. *h.* = hyphae; *o.* = ovary wall; *s.* = seed.  $\times 300$  diam.

Fig. 2. Cell from mesophyll (bundle sheath) of the cotyledon filled with hyphae. *h.* = hyphae; *v.* = vessel in median vascular strand; *chl.* = chloroplast. Camera lucida drawing from a section  $22\ \mu$  stained cotton blue in lactic acid. Leitz obj. 6; Zeiss compens. oc. 12.

Fig. 3. Mycelium of mycorrhizal Fungus outside root; hyphae continuous with those in cells. *r.* = root; *v.* = 'vesicles'.  $\times 330$  diam.

Fig. 4. Mycelium of mycorrhizal Fungus, from pure culture of same on *Calluna*-extract gelatine *v.* = 'vesicles'.  $\times 330$  diam.

Fig. 5. Transverse sections of successive leaves near apex of shoot. *g.* = groove on abaxial side; *s.* = air-space.  $\times 100$  diam.

Fig. 6. Longitudinal section of leaf parallel to upper surface. *c.* = crystals of calcium oxalate.  $\times 68$  diam.

Fig. 7. Coils of mycelium between adjacent leaves, penetrating the epidermis and mesophyll on either side. *e.* = epidermis; *m.* = mesophyll cell; *h.* = hypha. From longitudinal section of shoot, stained Benda hæmatoxylin.  $\times 540$  diam.

Fig. 8. Mycorrhizal Fungus. Hyphae and pycnidia from pure culture on rice. Six weeks. *p.* = pycnidium.  $\times 75$  diam.

Fig. 9. Mycorrhizal Fungus. Pycnidium more highly magnified after escape of pycnidiospores. *p.* = pycnidium; *s.* = pycnidiospore.  $\times 210$  diam.

Fig. 10. Normally infected seedling from unsterilized sand culture in tube as shown in Text-fig. 1. 282 days after planting.

Fig. 11. Root mycorrhiza. Superficial cell of young root with hyphae. Camera lucida drawing from whole root, stained cotton blue in lactic acid. Zeiss. hom. imm. 3 mm. Compens. oc. 12.  $\times 1620$  diam.

Fig. 12. Infection of leaf. Cells of the mesophyll with invading hyphae. *m.* = mesophyll cell of leaf; *h.* = hyphae; *chl.* = chloroplast. Camera lucida drawing from transverse section of shoot (Fig. 5). Zeiss. hom. imm. 3 mm. Compens. oc. 8.  $\times 880$  diam.

Fig. 13. Infection of leaf. Mycelium in air-space of leaf, with crystals of calcium oxalate. *C.* =  $\text{CaO}$ ; *h.* = hyphae. Camera lucida drawing from longitudinal section of a fresh leaf. Leitz obj. 6. Zeiss compens. oc. 12.  $\times 880$  diam.

Fig. 14. Sterile seedling growing in nutrient agar and artificially infected from a pure culture of the mycorrhizal Fungus. Ninety-five days from sowing; twenty-seven days after planting and inoculation. *f.* = limit of fungal growth in agar. This seedling subsequently reached a height of 6 cm.  $\times 1\frac{1}{2}$ .

Fig. 15 *a.* Sterile control seedling in nutrient agar. Ninety-five days from sowing; twenty-seven days after planting. No subsequent growth took place in this or other controls.  $\times 1\frac{1}{2}$ .

Fig. 15 *b.* Sterile control seedlings on filter-paper. Same age as Fig. 15 *a.*

Fig. 16. Sterile seedling, infected artificially from a 'strong' culture of the mycorrhizal Fungus in nutrient agar. Ninety-five days from sowing, twenty-seven days after planting. *h.* = mycelium growing out from shoot.

Fig. 17. Sterile seedlings on filter-paper, inoculated from a pure culture of the mycorrhizal Fungus supplied with distilled water only (cf. Fig. 18). Ninety-five days from sowing; twenty-one days after inoculation. *f.* = Fungus.

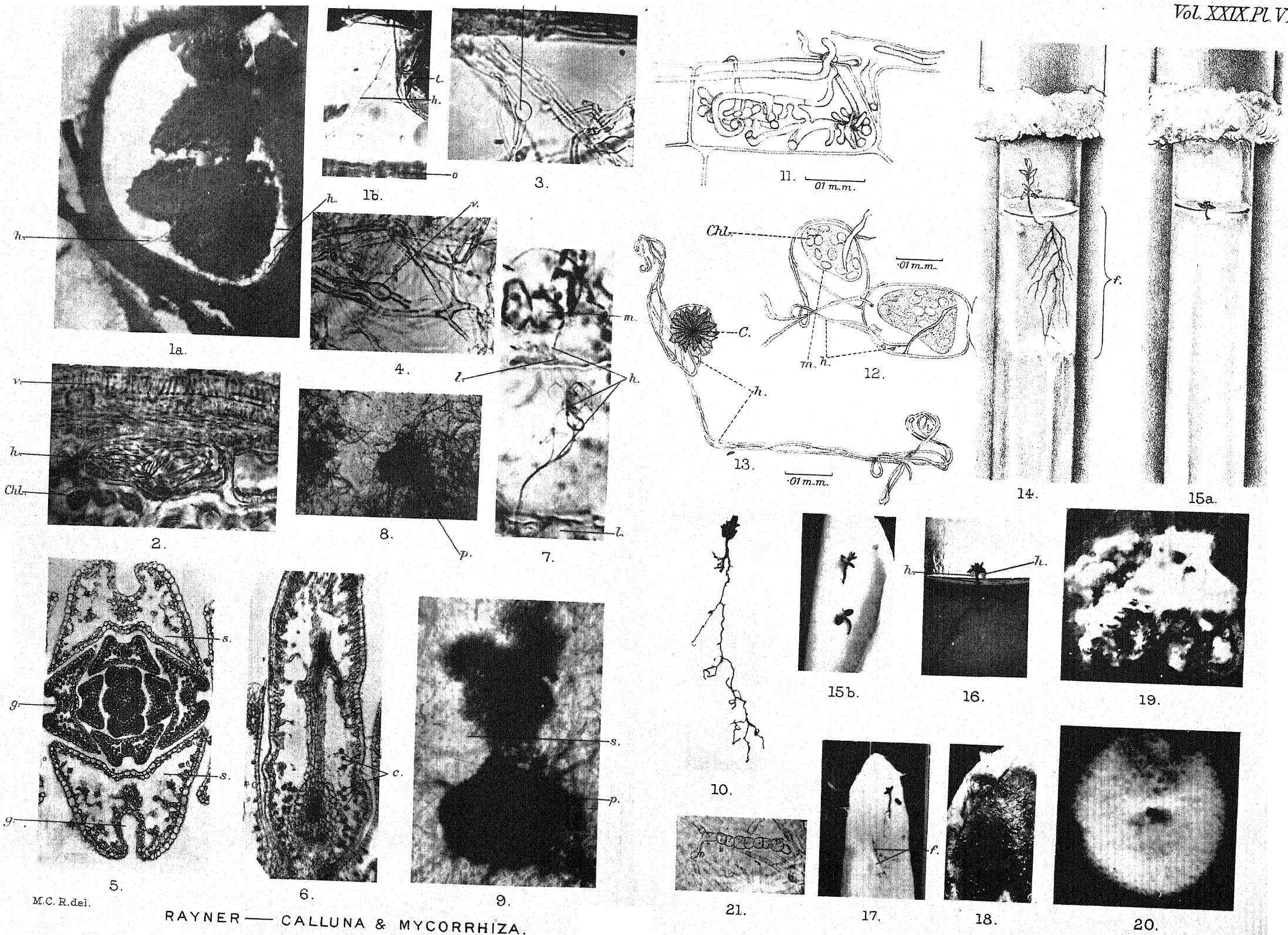
Fig. 18. Sterile seedlings of same age under similar conditions, but supplied with a dilute nutrient solution extract of distilled water. Seedling completely parasitized and destroyed; Fungus vigorous and forming pycnidia.

Fig. 19. Mycorrhizal Fungus. Pure culture on rice. Five weeks. Diameter 4.5 cm.

Fig. 20. Mycorrhizal Fungus. Pure culture on *Calluna*-extract gelatine. Fifteen days.

Fig. 21. Mycorrhizal Fungus. Mycelium from five-weeks' culture on nutrient agar.  $\times 250$  diam.

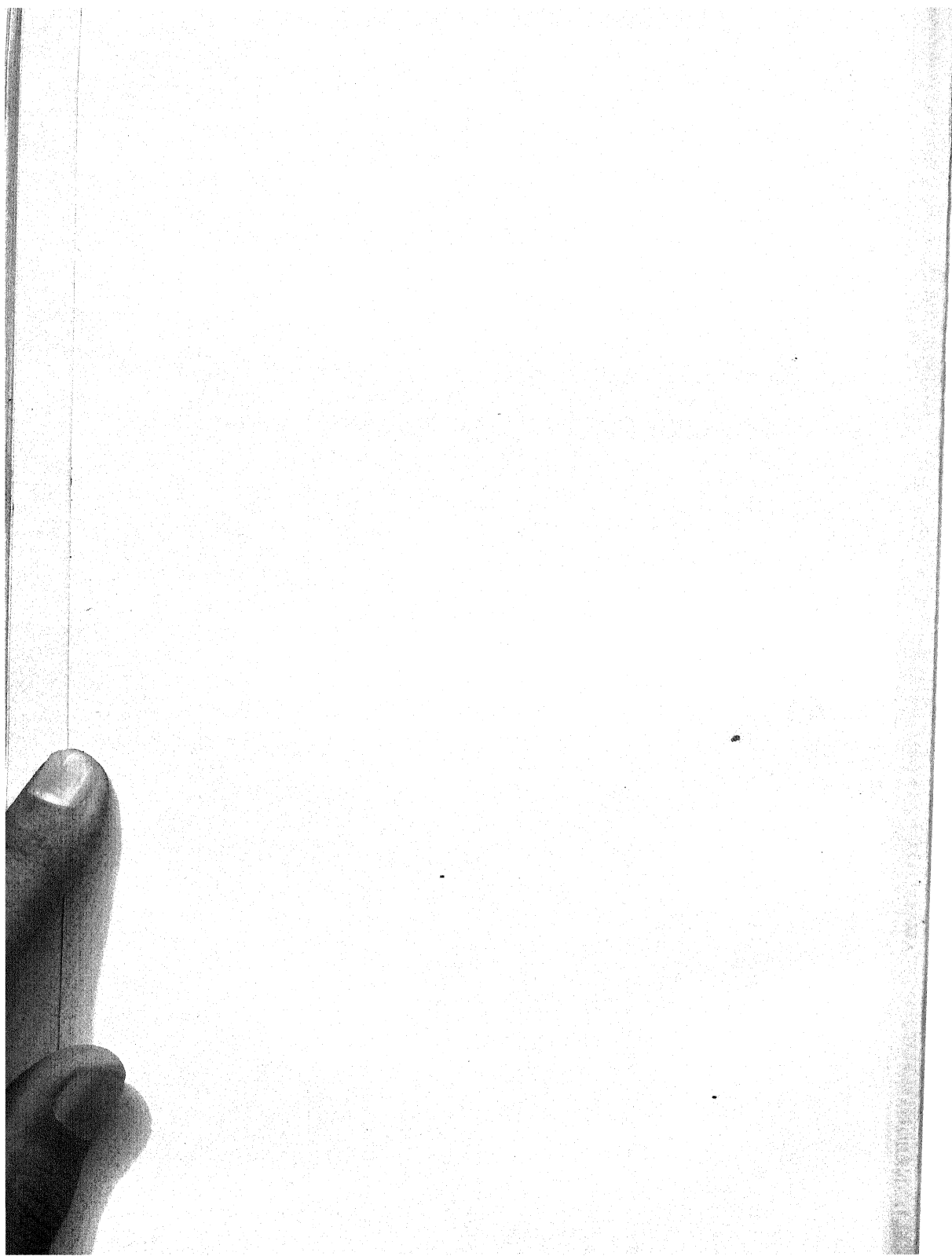




RAYNER — CALLUNA & MYCORRHIZA.

Huth, Lond.





# Observations on the Germination of the Spores of *Coprinus sterquilinus*, Fr.

BY

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With Plate VII.

**INTRODUCTION.** This work was originally undertaken in October, 1912, with the idea of investigating the nuclear phenomena in the genus *Coprinus*, but, owing to difficulties experienced in the germination of the spores, attention was subsequently centred on that. The observations recorded in this paper were made in the Botanical Department of the University of Bristol.

Work on coprophilous Fungi generally has been largely morphological and systematic, but a good deal of attention has been paid to the germination of the spores.

**Historical.** The opinion was held for many years that the spores of coprophilous Fungi would only germinate after having passed through the alimentary canal of an animal, but there is very little direct evidence on this point. Janczewski (11, pp. 257-62), 1871, attempted to germinate the spores of *Ascobolus furfuraceus* in nutrient solutions, but failed. He therefore fed rabbits with bread containing spores, and found germination had commenced when the dung was deposited. De Bary (7, pp. 375-7), 1884, germinated spores of coprophilous Phycomycetes—*Mucor*, &c.—in pure water, and was successful in germinating those of *Sordaria* and *Coprinus* in nutrient solutions. His attempts with *Ascobolus furfuraceus*, however, failed. Brefeld (2-4), 1891, was unable to germinate the spores of the latter genus, but he was very successful with those of various species of *Coprinus* and other Agaricineae. Masee and Salmon (14, 15), 1901, experimented with the spores of *Ascobolus perplexans* and *Ascobolus glaber*. The spores germinated in nutrient solutions at 80° F. after twenty hours, but at 60° F. only very feeble germination occurred after a much longer period. Attempts to germinate the spores of other species of *Ascobolus* and those of other Fungi failed. Falck (9, pp. 1-3), 1904, was unable

to germinate the spores of coprophilous Basidiomycetes. He passed some spores through the bodies of maggots to see if this would have any effect on their germination, but it did not. Blackman and Fraser (1, p. 355), 1906, failed to germinate spores of *Humaria granulata* in nutrient solutions, the same difficulty being experienced by Fraser (10, pp. 350-1) with the spores of *Lachnea stercorea*. Germination only took place after the spores had been passed through various digestive fluids. Schmidt (20, pp. 73-5), 1912, working on the propagation of the coprophilous Fungi, found in the case of many Phycomycetes and Ascomycetes that a certain temperature was necessary for the germination of the spores. Above this temperature (40°-42° C.) germination would only occur when certain chemical reagents were also used. He was unable to germinate the spores of some genera of Ascomycetes at all. From the above evidence it is seen that a general difficulty has been experienced in the germination of the spores of coprophilous Fungi.

*Material.* The material for the following work was obtained on cultures of horse-dung, which were set out at regular intervals to ensure a continuous supply in the laboratory.

The species of *Coprinus* used for these observations was submitted to Mr. A. D. Cotton, of the Kew Herbarium, for identification. He named it *Coprinus sterquilinus*, Fr., belonging to the 'comatus' section. The blackening of the apex of the stalk with age is a characteristic feature. The species possesses a ring, and before opening resembles a very slender comatus in form. The above is quoted from Mr. Cotton's description. This species only appeared during the winter months on cultures kept at 25° C. and 30° C., but it grew equally well at all temperatures from 15° C. to 30° C. throughout the summer.

*The germination of the spores.* Several attempts were made to germinate the spores in different nutrient solutions, those suggested by Küster (12, pp. 114-45) as particularly suitable for the germination of *Coprinus* spores being tried, but without success. Fraser's method of digesting the spores was then followed exactly as given below, and germination took place (10, pp. 350-1). Spores were placed successively in:

1. Saliva.
2. Gastric juice (a few drops of liquor pepticus of Benger in 0.2 per cent. aqueous solution of HCl).
3. Pancreatic juice (one part of Benger's liquor pancreatus to two parts of 1 per cent. aqueous solution of sodium carbonate).
4. Liquid extract of horse-dung.

The spores were left in each of the first three fluids for three hours, and in the last for fourteen to eighteen hours at 39° C. They were then placed in a temperature of 25° C.

Brefeld (2, pp. 14-16) was able to germinate the spores of *Coprinus*

*stercorarius* in liquid extract of dung without any trouble. He says: 'Sie keimen sofort, wenn ein Tropfen Nährlösung — Mistdecoct — sie umgibt; auch wenn die Sporen länger als ein Jahr trocken aufbewahrt sind, werden nach wenigen Stunden schon die Anzeichen der Keimung deutlich.' More attempts were therefore made according to Brefeld's method. Hanging-drop cultures were made continuously for several weeks in solutions of varying strengths and at different temperatures, but without success. Eventually the spores in one culture commenced germinating vigorously. These were stained on the coverslip according to the method devised by Overton (22, pp. 27-9). On staining, the mycelial tubes were found to be so thickly covered with Bacteria (Pl. VII, Fig. 10) that their contents were indistinguishable. The spores were again successfully grown in the same medium, but the Bacteria were always present. This suggested that perhaps the Bacteria played some part in the germination. Accordingly, part of the medium was sterilized by first passing it through a candle filter, and then heating it for two hours at 144° C. in the autoclave.

Hanging-drop cultures were made with and without the Bacteria. In those with the Bacteria, germination always took place within twenty-four hours, but it never occurred at all without them. These experiments seem to show that the Bacteria are in some way necessary for the germination of the spores. This is again borne out by the fact that they were also present in the cultures in which the spores had first been passed through digestive fluids.

*The Bacteria.* Brazilin was found to be a good stain for differentiating the Bacteria from the mycelium, but it gives no differentiation in the Bacteria themselves. They can, however, be detected under the microscope without staining. The Bacteria are very short rods measuring 0.8  $\mu$  by 1.2  $\mu$  in breadth and length. They occur in large numbers covering the mycelial tubes, particularly at those places where branching occurs, that is, at the centres of the greatest activity (Figs. 6, 7, 10). In such cases it is impossible to distinguish the tubes underneath. Occasionally the Bacteria occur in chains or in groups of three or four, but more often they are scattered evenly, there being as many as 4,000,000 to the square millimetre. Löhnis (13, p. 105) figures Bacteria in such groups and chains as common forms in manure from farmyards.

The Bacteria develop quite well without the spores in liquid dung decoction, but so far have not grown in beer wort. They seem to grow better in cultures where spores are present. This suggests some kind of interdependence between the two, but of exactly what nature it is difficult to ascertain. Many attempts have been made to isolate the Bacteria on solid media, but so far without much success. In view of the fact that the Bacteria are abundantly present in the liquid extract of dung, this result is very peculiar.

In some hanging-drop cultures of the Bacteria, rod-like forms appeared after about five days, and these always seemed to inhibit germination and development of the spores. The actual significance and function of these bacilli has so far not been worked out, owing to difficulties in staining and isolation. The characteristics of the two forms of Bacteria have not as yet been determined owing to the impossibility of isolating them in pure cultures.

Germination of the spores is most vigorous at 30° C., so that the combined influences of warmth and the Bacteria seem to react favourably on the spores, and lead to their development.

A few experiments were made on the connexion between the germination of the spores of *Mucor* and *Coprinus*, since the two never germinate together, the one which first commences to develop apparently preventing the other from doing so at all. *Mucor*, being thinner walled, &c., is usually the one to attain the dominance, since it responds more quickly to the stimulus of moisture, &c. Hanging-drop cultures of *Mucor* and *Rhizopus nigricans* were made with and without the Bacteria, and showed peculiar results. Germination of these spores only occurred when the Bacteria were absent. This is contrary to what would naturally be expected, since it does not seem probable that the Bacteria should prevent the development of the *Mucor* spores, as they must both be present in the dung from the beginning. If there is any interdependence between the *Coprinus* spores and the Bacteria, then it is possible that the latter only develop to their full extent when the former are present, and vice versa, so that the Bacteria may produce something not altogether favourable to the germination of the *Mucor* spores.

No reference can be found to any similar case of so close a connexion between the germination of Fungus spores and Bacteria. It is, however, a well-known fact that the spores of Myxomycetes will not germinate without Bacteria. Nadson (17, p. 37) has worked on the development of *Dictyostelium mucoroides*, Bref., and finds that certain Bacteria are of great benefit to the spores. Molliard (16) found that certain Bacteria aid the development of the perithecia in *Ascobolus*. He says: 'J'ai pu me convaincre que c'est bien à une association du champignon avec la bactérie qu'il faut rapporter la formation abondante et hâtive des périthèces.' He thinks these Bacteria assist the mycelium in producing 'une atmosphère confinée', which it is incapable of realizing alone. Falck (9, pp. 1-3), after passing the spores of certain Basidiomycetes through the bodies of maggots, found they were covered with Bacteria, and had on that account to be examined very carefully. He did not endeavour to find out whether the Bacteria had any influence on the germination, but it seems not unlikely that they would have reacted favourably on the spores, had they not been removed before he made any observations. Cutting (6, pp. 400-2) had great difficulty in

germinating the spores of *Ascophanus carneus*, Pers., but found that an alkaline medium combined with a certain temperature was necessary. He was unable to obtain pure cultures, growth proceeding only for a very short time, certain Bacteria, which were always present, seeming to him to stop growth, owing to their using up the oxygen. Wehmer (21, pp. 311-16) found it impossible to germinate spores of *Merulius*, although various methods were tried during a period of three years. He seemed to think that the spores are incapable of development, and that propagation takes place vegetatively. Falck (9, pp. 1-3) also thinks that among the innumerable spores that are formed by Basidiomycetes, only a few are able to develop. This does not seem the case in *Coprinus*, the difficulty here being more likely one of failure to obtain the right conditions for germination. These conditions must necessarily be hard to imitate for coprophilous Fungi, owing to the nature of their substratum and dissemination.

Molliard's explanation (16) quoted above can hardly be applied in the case of the germinating *Coprinus* spores and their accompanying Bacteria. Probably the Bacteria produce substances favouring the growth of the Fungus spores, whilst the activity of the *Coprinus* mycelium in its turn and in the same way benefits the development of the bacterial cells. About this there can in fact be no doubt. The actively growing Bacteria are found covering in large numbers the mycelial tubes of the Fungus, especially at points where branching occurs (Fig. 10). Possibly also in each case injurious by-products are formed by one of the two organisms which may be removed or rendered innocuous by the other. The fact that the presence of the longer bacilli inhibits the development of the *Coprinus* mycelium seems to point to the fact that in this case at any rate some toxic compounds are formed which are not removed.

Schmidt (20, pp. 73-5) finds that the combined influences of a fairly high temperature and certain chemical reagents will lead to germination in some cases. It seems not unlikely that the Bacteria may play the same part as these chemical reagents. In 1905 Duggar, in a paper on Mushroom growing and spawn-making (8, pp. 12-18), gave an account, in the section on Germination Studies, of Margaret Ferguson's work on the relation of stimuli on germination in certain species of *Agaricus*. From her results she concludes 'that the problems involved are not the well-known simple nutrient and physical factors'. Thousands of cultures were made in nutrient media, but germination was only erratic. When a bit of mycelium was introduced into the culture, almost a perfect percentage of germination was obtained. Duggar concludes the stimulus here to be of enzymatic nature, although perhaps it could only be looked upon as a substitution stimulus, and not one which would obtain in nature. It seems probable that the Bacteria have the same sort of influence as the living tissue in the case of *Agaricus campestris*, their influence being possibly of an enzymatic nature.

Brefeld has not worked on this particular species of *Coprinus*, and it is very likely that different conditions may be needed here for germination. In preparing his media, Brefeld (5, p. 32) never subjected them to a higher temperature than 80°–90° C. The Bacteria have to me proved very hard to kill, and the medium had first to be passed through a candle filter and then heated for two hours at 144° C., in order to completely sterilize it. Brefeld thought that heating above 90° C. altered the chemical constituents of the medium, but it may equally well have the effect of killing the Bacteria and in that way of rendering it unfit for germination purposes.

*Formation of the mycelium.* The spores of *Coprinus sterquilinus*, Fr., measure 0.15 mm.–0.18 mm. in length, and 0.008 mm.–0.012 mm. in breadth, and they do not seem mature until about three weeks after they have been shed; but if dried for two days at 40° C., they will germinate at once. This resting-period, which was also noticed by Falck (9, pp. 1–3), may be an adaptation on the part of the spores to retard germination until the substratum has become fairly dry and such Fungi as *Mucor* have disappeared.

The stages in the formation of the mycelium agree in the main with those figured by Brefeld (2, pp. 14–16) for other species of *Coprinus*. Fig. 1 shows the clear, light-refracting 'Bläschen' which first appears, and from which, in about two days, one or more germ-tubes are put out (Figs. 2–5). The vesicle never attains the size of the spore as described by Brefeld (2, pp. 14–16) for *C. stercorearius*, scarcely even becoming one-half as big (Figs. 2–5).

After two or three days a much-branched mycelium is formed, growth proceeding very quickly once germination has taken place. Fusions between neighbouring hyphae appear very abundantly after the fourth or fifth day. These fusions are characteristic of the mycelia of many Basidiomycetes. Brefeld (2, p. 19) has only figured fusions between hyphae of the same mycelium in *Coprinus stercorearius*, but he says: 'Nicht ganz ohne Interesse schienen mir Versuche zu sein, wie sich zwei verschiedene Mycelien zu einander verhalten möchten.' Fusions between hyphae, from different mycelia, which run closely parallel with one another, have often been observed in cultures of *C. sterquilinus* (Figs. 8 and 9). The significance of such fusions is difficult to determine. Brefeld (2, p. 19) suggests that those between hyphae of the same mycelium are for balancing the cells. The same idea may also hold for those between different mycelia, and the fusions may be an advantage when several spores are germinating in a confined space. Thus the different mycelia may help instead of hindering one another.

Many attempts have been made to obtain pure cultures of *Coprinus* on solid media, but without success.



### SUMMARY.

1. The spores of *Coprinus sterquilinus*, Fr., will not germinate *without Bacteria*.
2. These Bacteria are short rods, measuring  $0.8\ \mu$  in breadth and  $1.2\ \mu$  in length.
3. The Bacteria are most abundant towards the centre of the mycelium, and at those points where branching occurs.
4. Development of the Bacteria is better in cultures where spores are present and are germinating.
5. The presence of certain longer bacilli prohibits development of the mycelium.
6. Germination is most vigorous at  $30^{\circ}\text{C}$ ., and does not occur at all below  $20^{\circ}\text{C}$ .
7. Fusions between neighbouring hyphae of different mycelia frequently occur.

### CONCLUSION.

It seems from the above results that these Bacteria are necessary for the germination of the spores of *Coprinus sterquilinus*, Fr., but in exactly what way they are of benefit to the spores is still matter for conjecture. Certainly they aid the spores in some way, and the question arises as to whether the spores are of any benefit to the Bacteria. From the way in which they cover the mycelial tubes this would seem to be the case. Nadson (18, p. 38), fully recognizing the importance of pure cultures, yet thinks that in some cases development is better where certain micro-organisms are also present. This theory is quite tenable if applied to the case of *C. sterquilinus*, since many Bacteria pass through the animal with the spores, so that their presence in artificial cultures may more closely approximate the conditions to those in nature.

The writer wishes to take this opportunity of expressing her thanks to Dr. O. V. Darbishire, under whose helpful direction and advice this work has been carried on.

THE UNIVERSITY,  
BRISTOL.

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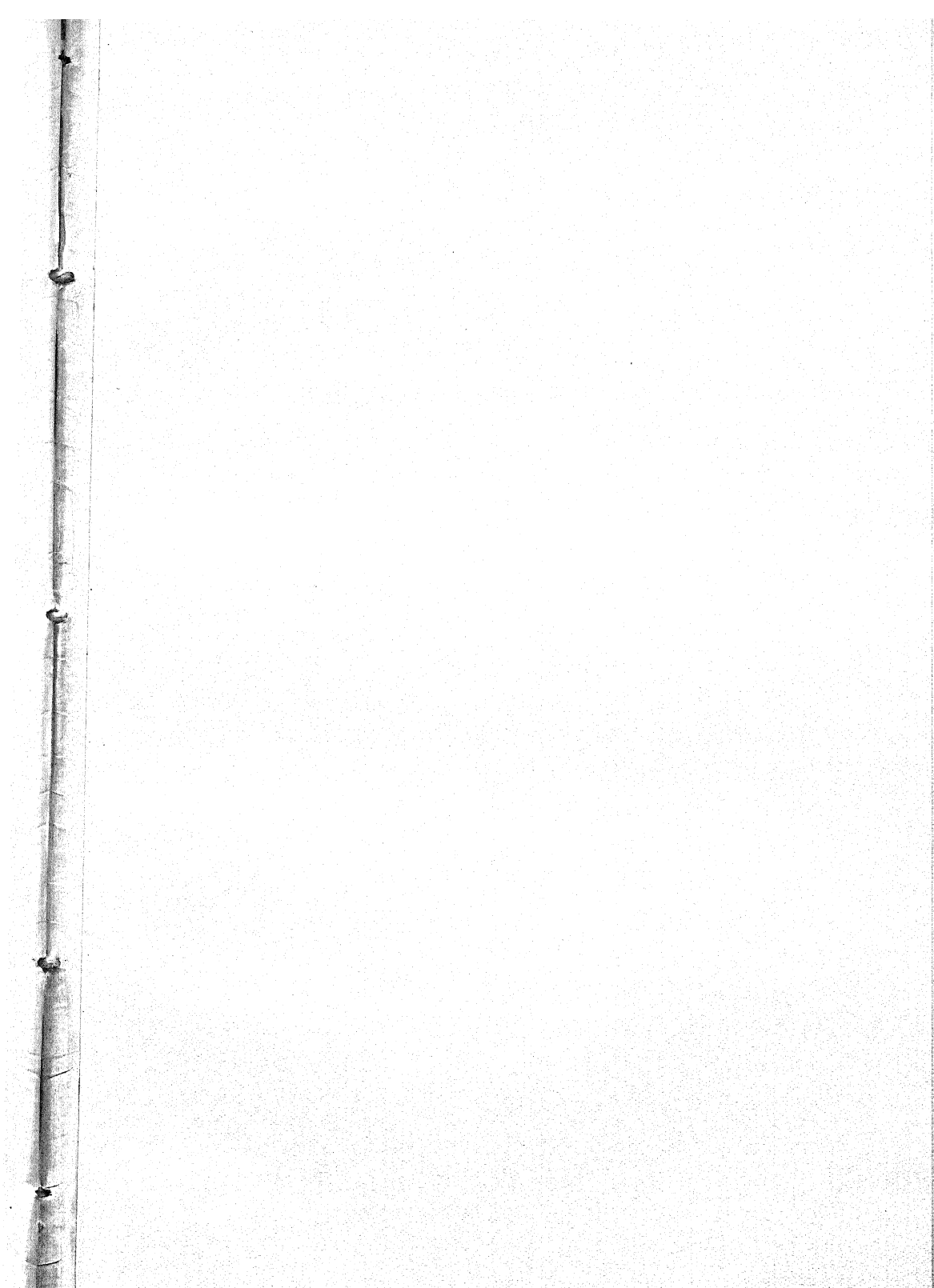
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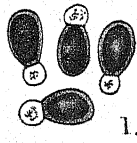
## EXPLANATION OF PLATE VII.

Illustrating Miss Baden's paper on Germination of the Spores of *Coprinus sterquilinus*, Fr.

(All the figures are drawn with the Abbé drawing apparatus. Figs. 1-5, 9, and 10 are drawn without the Bacteria, for the sake of clearness. Figs. 6 and 7 on a lower scale.)

- Fig. 1. Germination of spore, showing clear, light-refracting vesicle (Leitz, obj. 7, oc. 4).
- Fig. 2. Germ-tubes arising from vesicle (Leitz, obj. 7, oc. 4).
- Fig. 3. Showing Fig. 2 more advanced (Leitz, obj. 7, oc. 4).
- Fig. 4. Germinating spore after about thirty-six hours (Leitz, obj. 7, oc. 4).
- Fig. 5. Ditto, after forty-eight hours (Leitz, obj. 7, oc. 4).
- Fig. 6. Germinating spore in early stages, thickly covered with Bacteria (Leitz, obj. 3, oc. 4).
- Fig. 7. Shows a slightly older stage than Fig. 6, the germ-tubes being covered with Bacteria (Leitz, obj. 3, oc. 4).
- Fig. 8. Cross-fusion between different mycelia at  $x$ , showing open connexion (Leitz, obj. 7, oc. 4).
- Fig. 9. Neighbouring mycelia, A, B, and C from different spores, connected together by cross-fusions at  $x_1$  and  $x_2$  (Leitz, obj. 3, oc. 2).
- Fig. 10. Mycelium covered with Bacteria, particularly towards the centre and at those points where branches are given off (Leitz, obj. 7 oc. 2).

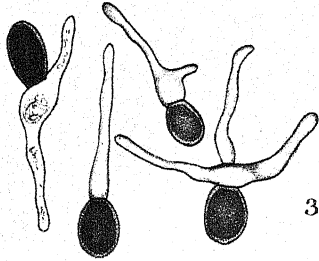




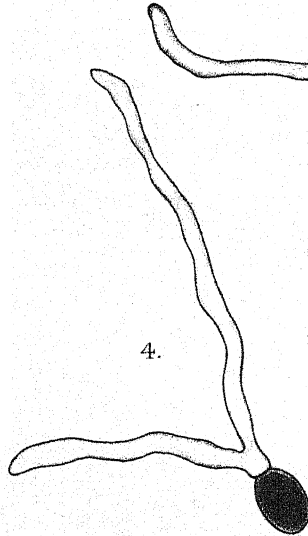
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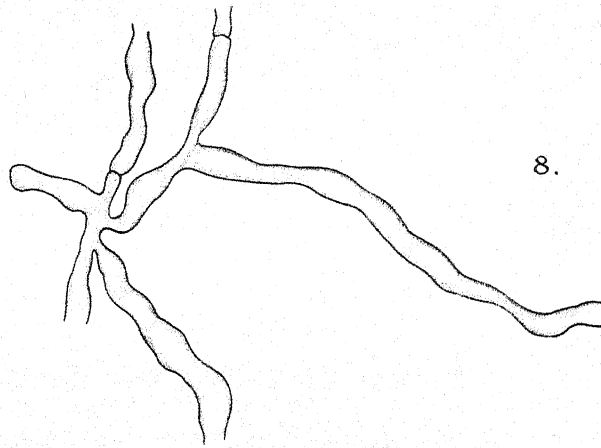
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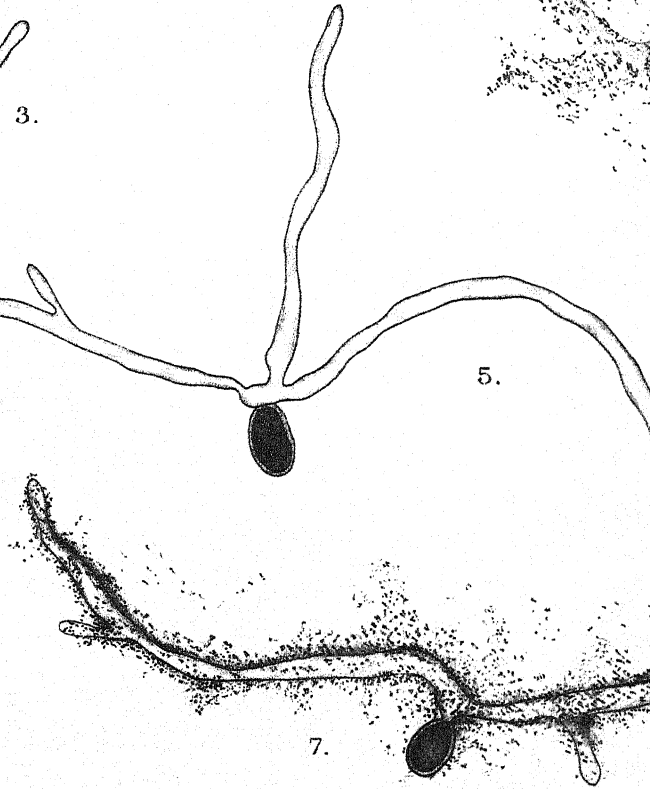
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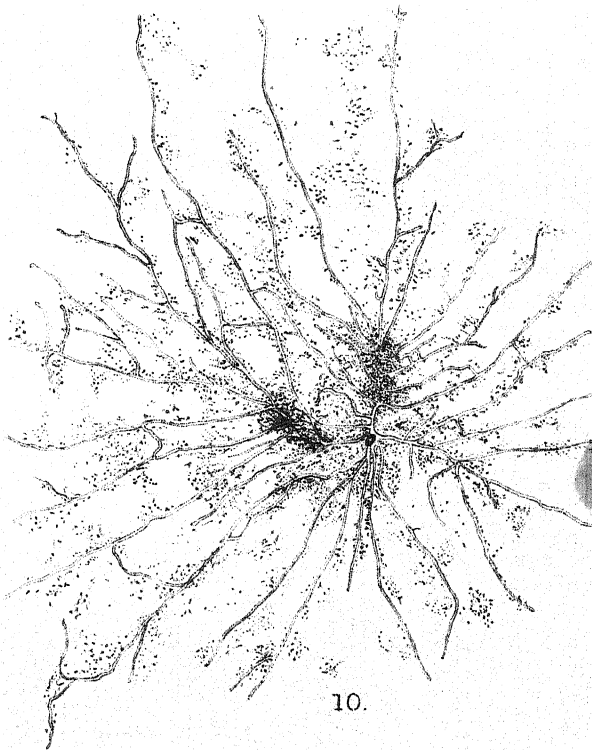
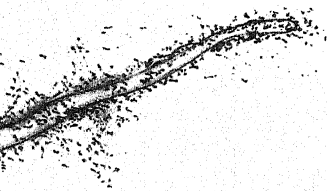
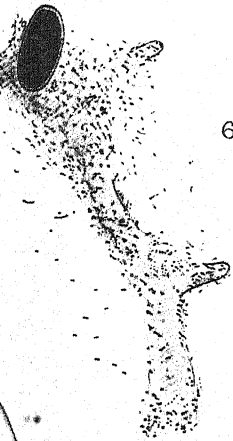
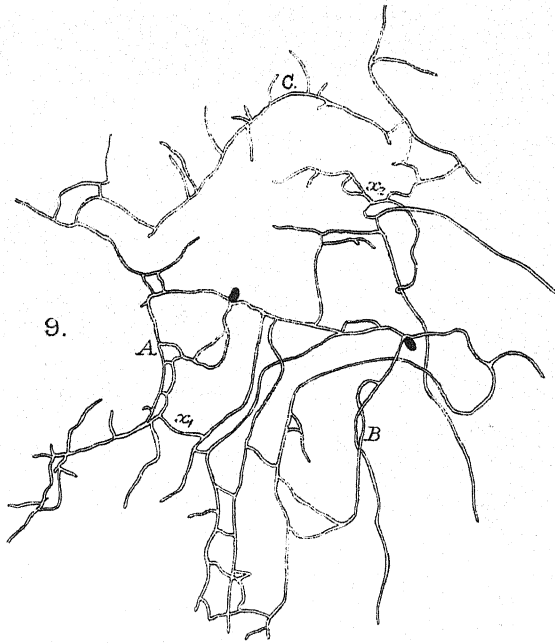
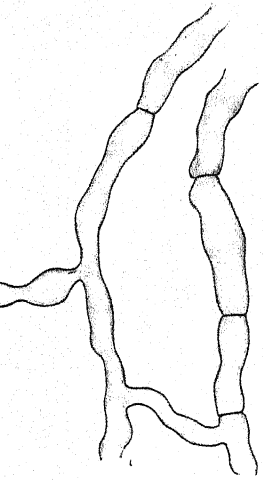


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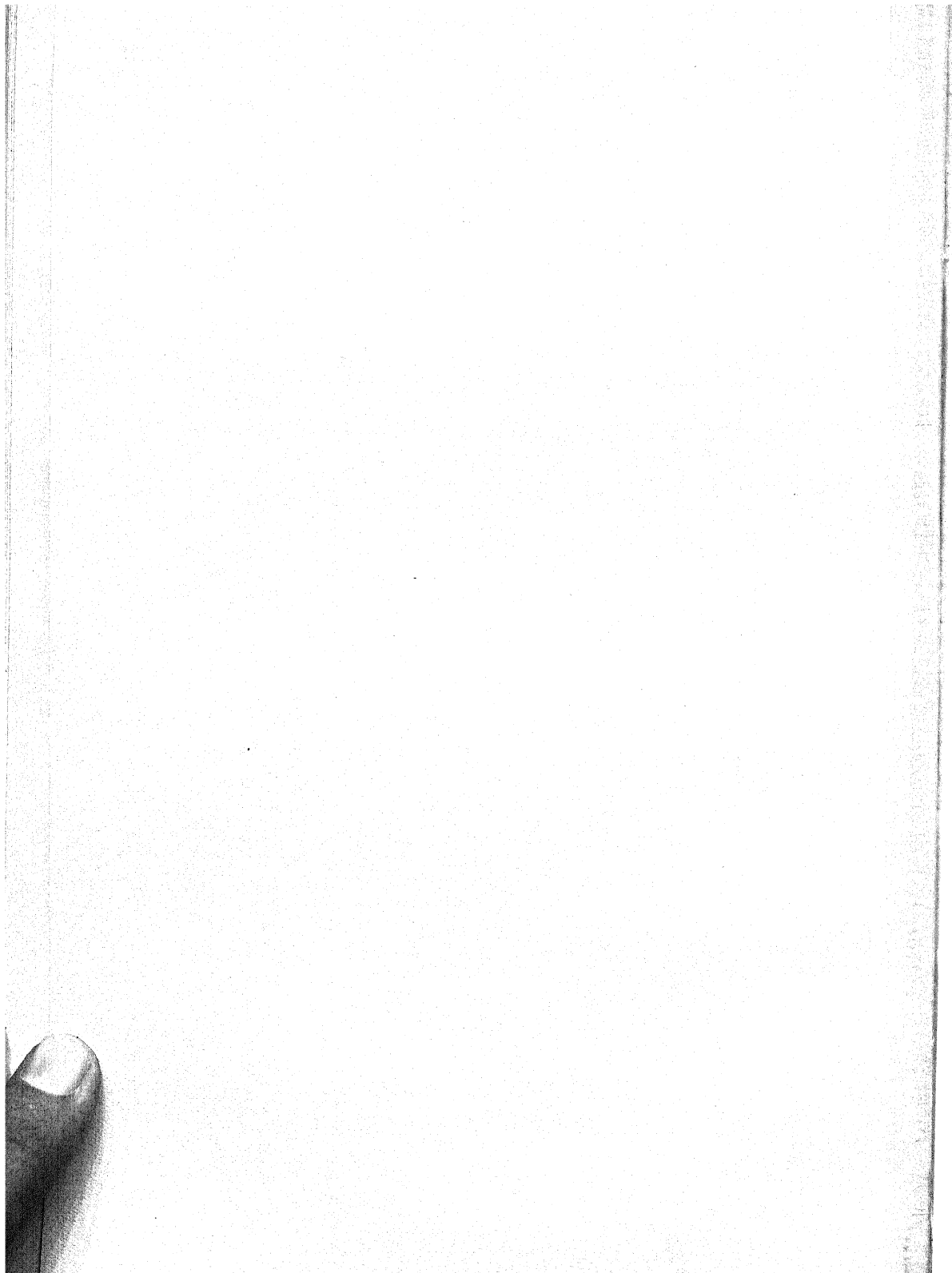
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M.L. Baden, del.

BADEN — COPRINUS.



Huth, lith. et imp.



# The Effect of Salt on the Growth of *Salicornia*.

BY

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With Plate VIII and four Diagrams in the Text.

CERTAIN plants grow in soils that contain a considerable percentage of mineral salts. Sodium chloride is the most common of these salts and is the one most widely distributed. In this country the land impregnated with salt is only found near the coast in such a position that it is periodically covered by sea water. These areas are known as salt marshes and are covered by a very characteristic vegetation. The plants that form this vegetation have always aroused considerable interest, as their habit is obviously different from that of the typical mesophytic plant. The difference of appearance is due to the marked succulence of the majority of the species inhabiting the salt marshes. As early as 1876 Batalin<sup>1</sup> correlated this succulence with the presence of salt in the soil, as he found that 'salt plants' cultivated under ordinary conditions in the botanical gardens of St. Petersburg lost their usual characteristics, but if they were treated with solutions of sodium chloride they developed normally. At a later date Batalin<sup>2</sup> made cultivation experiments with plants of *Salicornia herbacea*, L., watering them with various salt solutions, and he found that the typical soft and fleshy habit was developed only when sodium chloride was present.

More recently the experiments of Lesage<sup>3</sup> have shown that ordinary non-succulent plants, e.g. *Lepidium sativum*, tend to become fleshy when cultivated in soil watered with solutions of sodium chloride. It is therefore probable that the presence of salt in the tissues of a plant has such an influence on the whole physiology of the plant as to alter its general structure, producing as one of the results the characteristic succulence seen in salt marsh plants.

The number of plants composing the vegetation of a salt marsh is

<sup>1</sup> Batalin, A. : Cultur der Salzpflanzen. Regel, Gartenflora, 28, 1876.

<sup>2</sup> Batalin, A. : Wirkung des Chlornatriums auf die Entwicklung von *Salicornia herbacea*, L. (Bull. du Congrès international de bot. et d'horticulture. St. Petersburg, 1886). Ref. in Bot. Centralbl. xxvii, 1886.

<sup>3</sup> Lesage, M. Pierre : Recherches expérimentales sur les modifications des feuilles chez les plantes maritimes. Revue générale botanique, t. ii, 1890.



comparatively small, and of this number the various species of *Salicornia* and *Suaeda* occupy the most exposed positions, and may perhaps be regarded as among the most typical of the plants. They are very succulent and contain a considerable amount of sodium chloride in their tissues. It seems to follow from the culture experiments of Batalin that at least one of these plants, e.g. *Salicornia herbacea*, L., has become so specialized as to require sodium chloride for its normal development, though it can grow in ordinary soils when freed from the competition of other plants.

The effect of salt on the growth of plants is somewhat uncertain. Lesage<sup>1</sup> found that the height of plants of *Lepidium sativum* decreased with the increase of sodium chloride in the soil, while Stange<sup>2</sup> found that plants treated with certain salt solutions, e.g. of potassium nitrate, also decreased in height. With regard to the salt marsh plants themselves, Ganong,<sup>3</sup> describing the plants found on the Bay of Fundy marshes, says of *Salicornia herbacea*, L., that the plants varied 'in size inversely with the saltiness of the habitat'. It was in the endeavour to obtain more information of the effect of sodium chloride that, some years ago at the suggestion of Professor F. W. Oliver, I made some experiments on the growth of these plants. I would like here to thank Professor Oliver for suggesting this work to me, and also for his kindness in bringing the seedling plants from the Bouche d'Erquy and for taking the photograph from which Pl. VIII was made.

The experiments were made with *Salicornia* and *Suaeda* seedlings, which were cultivated in the presence of various amounts of sodium chloride. In this way the effect of different amounts of salt on the growth of the plants was ascertained.

The experiments were of two kinds and may be considered under the two following divisions:

I. Seedlings cultivated on their natural soil, and treated with solutions containing various percentages of Tidman's sea salt.

II. Seedlings cultivated in nutritive solutions to which definite quantities of sodium chloride had been added.

#### I.

Pieces of turf were brought from the Bouche d'Erquy in Brittany. The turf was composed chiefly of seedlings of *Salicornia ramosissima* or *Suaeda maritima* and of *Glyceria maritima*. The turf had been obtained from different parts of the marsh, so that some pieces bore *Salicornia* seedlings and *Glyceria*, while others had *Suaeda* seedlings and *Glyceria*. Sods were cut from the turf so that they just fitted into shallow porous earthenware

<sup>1</sup> Lesage, loc. cit.

<sup>2</sup> Stange, B.: Beziehungen zwischen Substratconcentration, Turgor und Wachstum bei einigen phanerogamen Pflanzen. Bot. Zeit., 50, 1892, p. 349.

<sup>3</sup> Ganong, W. F.: Vegetation of the Bay of Fundy Marshes. Bot. Gaz., vol. xxxv, 1903, p. 357.

pans. The pans were divided into four sets of six, two (A and B) with seedlings of *Salicornia ramosissima*, and two (C and D) with seedlings of *Suaeda maritima*. In each lot each pan was treated differently, and was watered with a solution containing one of the following quantities of Tidman's sea salt, 0%, 1%, 2%, 3%, 4%, or 5%. Solutions of Tidman's sea salt were used so that the plants should be exposed to an influence resembling, as far as possible, that of sea water.

The sods were cut near the end of April when the seedlings were very young. These were apparently all at the same stage of development, their cotyledons were expanded, but no epicotyls were visible above the level of the cotyledons. The experiments were begun on April 28 and were continued till June 25. The plants were exposed to the different degrees of salinity by immersing the pans in the various solutions for a period of two hours or longer every seven days or so. If the soil became dry between the immersions, the pans were watered with equal volumes of the appropriate solutions.

It was soon evident that the rate of growth of *Salicornia* and *Suaeda* differed in the various pans, and this difference became increasingly evident as the treatment was continued. Certain plants, five in number, were chosen in each pan, and the rate of growth of their epicotyls noted by measurements taken at intervals. But, as there was found to be considerable variation in the height of the plants in the same pan, the curves of growth so obtained are not of sufficient value to warrant their reproduction here. The sods were untouched before the treatment began, the seedlings were allowed to remain as the seeds had fallen and germinated, so that they were not at all evenly distributed. It was thought therefore that the variation in the height of the individual plants in the same pan was due to the competition of the plants with one another, and with the *Glyceria* which was also present. In spite of this variation in size of some of the plants, the effect of the various degrees of salinity on the growth of the plants as a whole was very evident, and is best shown by a comparison of the average height obtained by the plants in the different pans. On June 1, nearly five weeks after the treatment was begun, the length of the epicotyl of each plant was measured and the average for each pan calculated. The figures obtained are given in the table below:

TABLE I.  
Average height of plants in millimetres.

| Tidman's<br>sea salt. | <i>Salicornia ramosissima</i> . |      | <i>Suaeda maritima</i> . |      |
|-----------------------|---------------------------------|------|--------------------------|------|
| %                     | A                               | B    | C                        | D    |
| 0                     | 8.2                             | 9.7  | 11.7                     | 10.8 |
| 1                     | 11.9                            | 12.7 | 12.4                     | 9.9  |
| 2                     | 5.5                             | 7.3  | 10.0                     | 8.0  |
| 3                     | 4.4                             | 3.7  | 8.0                      | 6.2  |
| 4                     | 2.3                             | 3.0  | 4.1                      | 2.3  |
| 5                     | 2.3                             | 3.2  | 4.7                      | 2.6  |
|                       | L                               |      |                          |      |

These results are represented graphically in Diagram 1, where the variation in height is plotted against the variation in salinity.

This diagram shows clearly that plants of *Salicornia* flourished best in the presence of a certain amount of salt, though growth was retarded if the

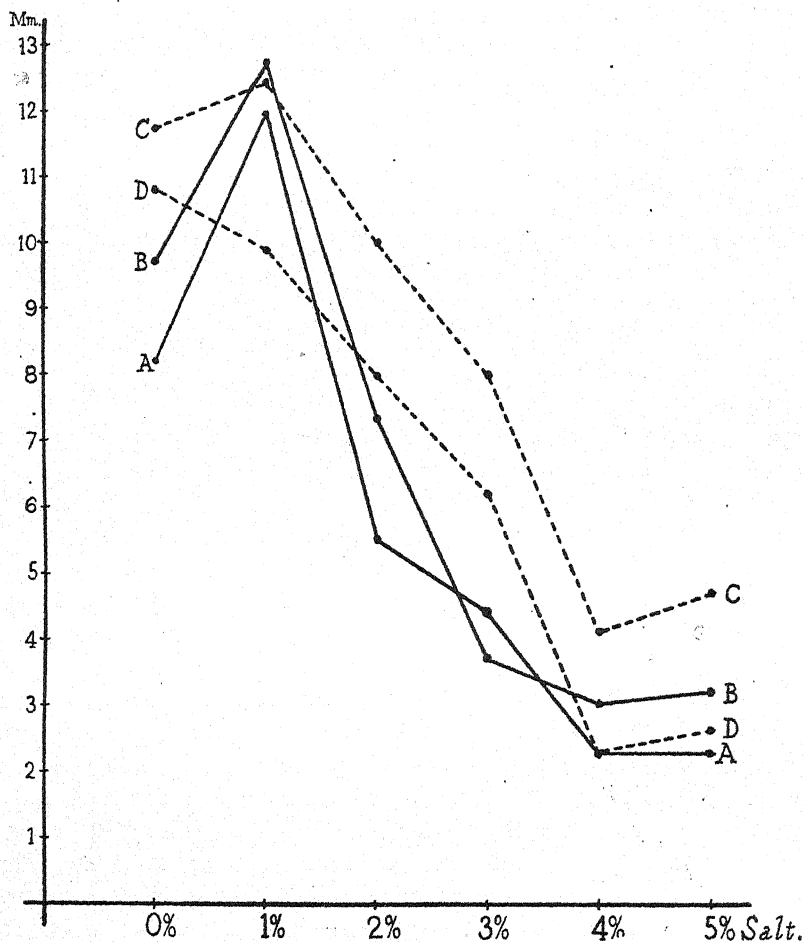


DIAGRAM 1.

Effect of degree of salinity on growth of *Salicornia* and *Suaeda*.

— *Salicornia ramosissima*, pans A and B.

- - - *Suaeda maritima*, pans C and D.

amount of salt present increased beyond a certain limit. In these experiments the greatest growth took place in the pans treated with the 1% solution of Tidman's sea salt. The effect of salt on the growth of *Suaeda maritima* is not so clearly shown, as in one case it slightly increased the growth and in the other slightly decreased it. The plants were measured again on June 16, when the same variation in growth was found. A number

of the *Salicornias* in the pans treated with the 1 % solution of Tidman's sea salt and with distilled water were measured again on June 25, when it was found that the difference of growth was maintained. The average height of the plants was 39.2 mm. in the first case, while it was 29.9 mm. in the second.

The effect of salt on the growth of *Glyceria maritima* was also noted. In the pans treated with the higher percentages of salt, 3 %, 4 %, and 5 %, it was found that after a time the plants turned yellow and finally died, while growth was greatest in the pans treated with water containing no salt. On June 25 the average height of the grass in each pan was obtained, and the decrease in growth according to the increase in salinity is shown in Diagram 2. Here the dotted lines represent the height of the *Glyceria* in the four sets of pans, A, B, C, and D, while the unbroken line gives the average height of the *Glyceria*.

It seemed to follow from these experiments that *Salicornia ramosissima* grew best when it was treated with a somewhat dilute solution of Tidman's sea salt (1 %), a considerably weaker solution than that of sea water. The other two salt marsh plants experimented with were less tolerant of salt. *Suaeda maritima* grew as well without salt as with it, while *Glyceria maritima* flourished much better in its absence. The fact that the plants were grown in soil made it somewhat difficult to be certain what was the actual salinity of the water available for the plant. When the experiments were begun, as the pans were comparatively small, it was thought that by immersing them for some time in the various solutions the excess of salt in the soil would be washed out, and that the salinity of the water in the soil would be approximately the same as that of the solution in which the pans were immersed. It was obvious that there was a certain variability in this salinity as, between the periods of immersion, water was lost from the soil by evaporation and through the transpiration of the plants. In order to ascertain the amount of this variation estimations were made of the salinity of the soil water in certain of the sods before and after immersion. The salinity was obtained by estimating the amount of water in the soil and the amount of chloride present, and then calculating the amount of salt in 100 c.c. of water, the chlorides being calculated as sodium chloride. Portions of soil were taken from one set of pans on June 5, just before the pans were immersed in the various solutions. The period of immersion lasted two hours and a half, then the pans were allowed to drain; a second lot of samples was taken on the morning of June 6.

The results of these analyses brought out the fact that not only was there a considerable variation in the salinity of the water of the soil in each pan, but also that this salinity was much greater than that of the solutions with which they were watered. It was found that the excess of salt was not washed out during the periods of immersion, but accumulated in the

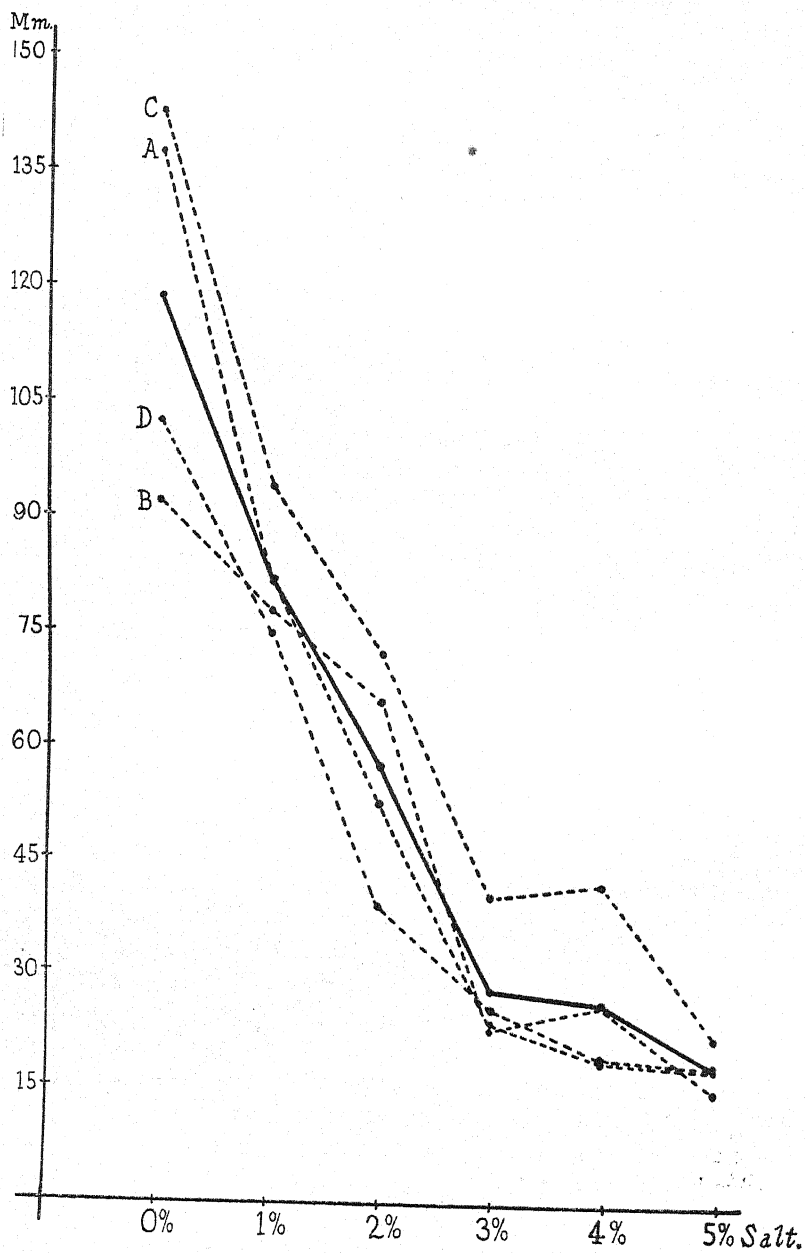


DIAGRAM 2.

Influence of salt on growth of *Glyceria maritima*.

--- Average height of plants in pans A, B, C, and D.  
 — Average height of all plants.

soil so that the salinity of the water available for the plants in the different pans was much greater than was supposed. The results obtained are tabulated in Table II, and the figures give some idea of the range of salinity in the various pans:

TABLE II.

*Range of salinity of water in soil.*

| Strength of solution of<br>Tidman's sea salt. | Salinity of water in soil.  |                            |
|---|-----------------------------|----------------------------|
|   | June 5<br>before immersion. | June 6<br>after immersion. |
| %   | %                           | %                          |
| 0   | 1.4                         | 0.6                        |
| 1   | 5.6                         | 2.5                        |
| 2   | 9.9                         | 5.7                        |
| 3   | 17.8                        | 10.5                       |
| 4   | 18.8                        | 12.1                       |
| 5   | 15.0                        | 11.2                       |

It is evident from these figures that the pans watered with the 1% solution, in which the greatest growth of *Salicornia* took place, contained soil the salinity of which varied within rather wide limits; in the sample analysed the variation was from 2.5% to 5.6%.

These experiments with plants grown in soil, while they show that plants of *Salicornia ramosissima* grow best in the presence of salt, do not show what percentage of salt is the most favourable, whether it is that which prevails in the normal habitat of the plants—approximately that of sea water—or not. The following year a series of water cultures was started to ascertain the effect of various amounts of sodium chloride on the growth of these plants.

## II.

Various attempts were made to cultivate plants from seed brought from the salt marsh, but these were all unsuccessful; the seeds germinated, but the majority of the seedlings died before they had attained any size. Successful cultures were made with young seedlings brought from the Bouche d'Erquy. Three sets of cultures were started with the following plants: (1) *Salicornia oliveri*, (2) *Salicornia ramosissima*, and (3) *Suaeda maritima*. The plants were grown in glass jars containing nutritive solution, made up according to Sach's formula, with the addition of various amounts of sodium chloride. Six variations in the amount of sodium chloride were used, the solutions in the jars containing respectively 0%, 1%, 2%, 3%, 4%, and 5% of this salt. Care was taken that all other conditions for growth should be as far as possible equal for all the plants. It was hoped in this way to observe the effect of the different concentrations of sodium chloride on the growth of these three plants.

The cultures were started when the seedlings were quite young, their cotyledons were expanded, but no epicotyls were visible. Seedlings were

chosen as far as possible of the same size, and six were placed in each culture jar. The experiments were begun about the end of April.

The growth of the plants in the various solutions was compared by measurements of the lengths of the epicotyls. These measurements were begun on May 8 and were continued till the beginning of July. Here, as in the previous experiments, the results obtained for *Salicornia* differed from those obtained for *Suaeda*. It was found that the increase in length of the plants of *Suaeda* was approximately equal in the nutritive solution without the addition of sodium chloride and in that to which 1% of this salt had been added, the total growth per plant in the first case being 112 mm. and in the second 110.2 mm. In the other jars the growth of the plants diminished as the amount of salt increased.

Plants of the two species of *Salicornia* grew much better in the presence of sodium chloride than in its absence. *Salicornia ramosissima* grew to approximately the same height in the jars with 2% and 3% of sodium chloride, and grew better in these than in the higher concentrations, while *Salicornia oliveri* grew rather more quickly in the solution containing 2% of sodium chloride than in the other solutions.

The cultures were on the whole more successful with *Salicornia oliveri* than with *S. ramosissima*, probably because the seedlings of the former were more easily transferred without injury to the culture solutions, as the seeds had germinated naturally in sand. The results obtained with *S. oliveri* are therefore selected to be given in detail.

Table III gives the average increase in height of the plants of *S. oliveri* and shows how this was affected by the variation of the salinity. These averages were calculated from the measurements taken of the lengths of the main axis of the epicotyls of the six plants grown in each jar.

TABLE III.

| Date.                         | <i>Salicornia oliveri</i> , Moss.<br>Growth in mm., in different concentrations of NaCl. |      |      |      |      |      |
|-------------------------------|--|------|------|------|------|------|
|                               | 0%   | 1%   | 2%   | 3%   | 4%   | 5%   |
| May 8                         | 2.4  | 3.3  | 3.0  | 2.9  | 1.6  | 0.9  |
| " 15                          | 3.3  | 4.9  | 5.4  | 3.8  | 2.1  | 0.6  |
| " 22                          | 1.9  | 3.4  | 3.5  | 3.6  | 2.3  | 0.6  |
| " 29                          | 1.5  | 4.7  | 5.3  | 4.7  | 2.8  | 1.0  |
| June 5                        | 1.1  | 4.0  | 5.6  | 4.4  | 3.3  | 2.1  |
| " 12                          | 0.7  | 3.4  | 5.7  | 4.6  | 3.2  | 1.6  |
| " 20                          | 0.5  | 3.4  | 4.9  | 4.4  | 3.7  | 1.6  |
| " 27                          | 1.5  | 3.9  | 5.5  | 5.0  | 4.3  | 2.6  |
| July 3                        | 0.3  | 3.4  | 4.7  | 3.9  | 3.3  | 2.0  |
| Growth in length<br>by July 3 | 13.2   | 34.4 | 43.6 | 37.3 | 26.6 | 13.0 |

These dates and figures are plotted in Diagram 3, and give a series of growth curves for the different degrees of salinity. From these curves it can be seen that from the beginning of the experiment growth was greatest



when sodium chloride was present, and that the greatest growth took place when plants were grown in the solution containing 2% of sodium chloride. The curves also show that increase of salinity beyond a certain concentration is unfavourable to growth, it being slowest in the solution containing 5% of sodium chloride.

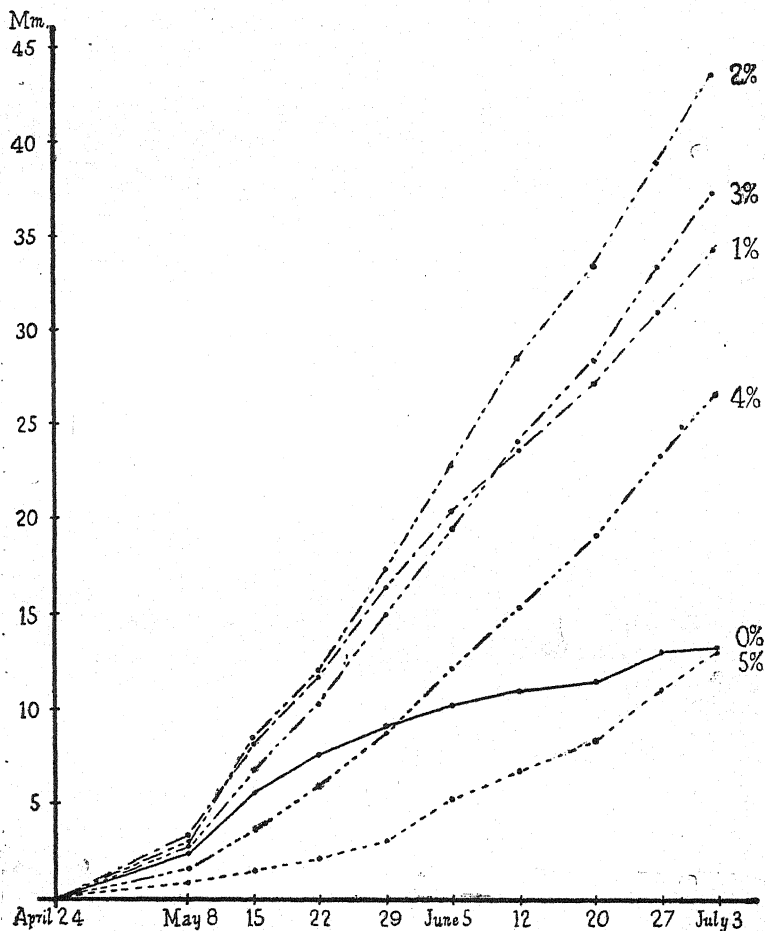


DIAGRAM 3.  
Growth of *Salicornia oliveri*, Moss., in nutritive solutions containing various percentages of sodium chloride.

— 0% NaCl.    - - - - 1% NaCl.    - · - · - 2% NaCl.  
- · - · - 3% NaCl.    - · - · - 4% NaCl.    - - - - 5% NaCl.

Many of these plants of *S. oliveri* branched; when this was the case the branches were also measured. It was found that the growth of the branches corresponded to the growth of the main axis, that is, that less growth took place in the absence of sodium chloride than when it was

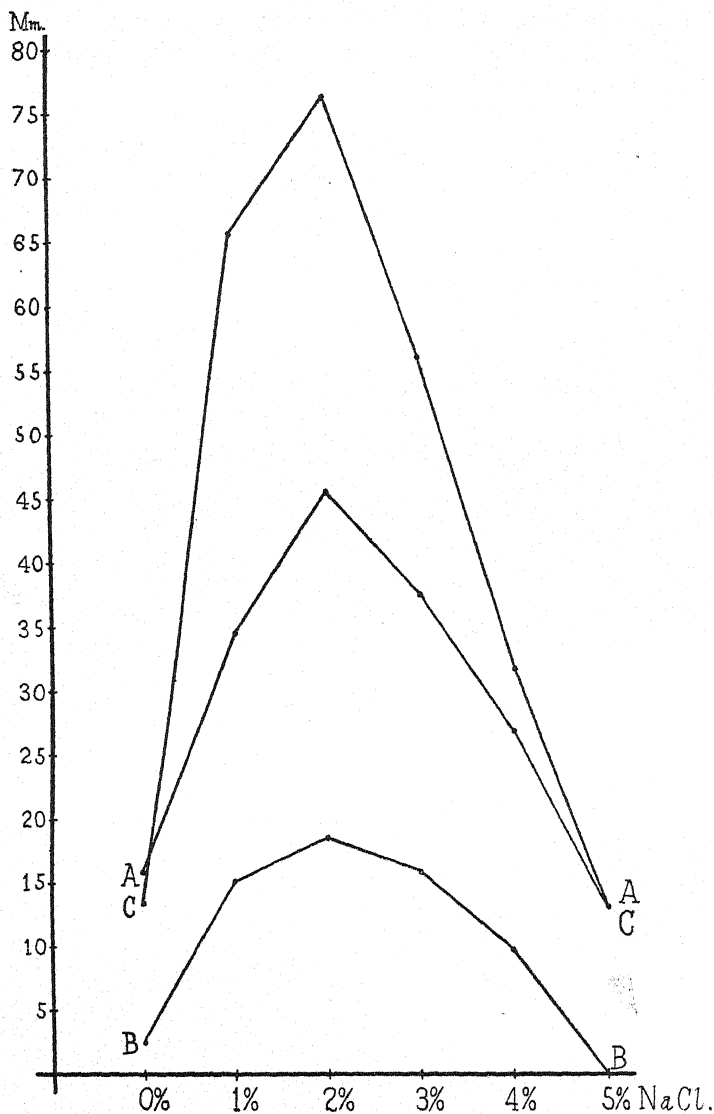


DIAGRAM 4.  
Effect of NaCl on growth of *Salicornia oliveri*, Moss.  
A. Average height of plants on July 3.  
B. Average length of branches.  
C. Average total growth per plant.

present in moderate quantity, and most when the solution contained 2% of this salt.

The measurements were discontinued on July 3, and the effect of the various degrees of salinity on growth till that date are shown graphically in

Diagram 4. This diagram gives three curves, A representing the average height which the main axes of the plants attained in the different solutions, B representing the average length of all the branches produced by the plants, and C the average total growth in length per plant (main axis and branches) under the various conditions of salinity.

The photograph from which Pl. VIII was made was taken at an earlier date, June 10, and shows the plants as they were then. The black paper surrounding the jars had been temporarily removed to show the development of the roots. It will be noticed that the root development is abnormally great, and that it also is affected by the amount of sodium chloride present in the solution.

After the photograph was taken the amount of sodium chloride present in each solution was estimated, and it was found that the amount of the salt present in each solution was approximately the same as at the commencement of the experiment, though the 2 % solution had become slightly more concentrated. The jars were at this time refilled with fresh solutions.

It is perhaps worthy of record that the few measurements that were made of the diameter of the internodes show that this was less in those plants grown without salt than in those grown with it. In those plants of *S. ramosissima* grown without salt the average diameter of the lowest internode of the plants on June 20 was 2.7 mm., while it was 3.5 mm. in those plants grown with 2 % and with 3 % of sodium chloride. These results are in accord with those of former experiments, showing that the presence of salt increases the succulence of plants.

It is also worthy of record that plants of *S. oliveri* grown without sodium chloride did not flower, while those with sodium chloride did. Two plants in the 3 % solution, three in the 4 % solution, and one in the 5 % solution flowered. This is probably the reason that the curve of total growth of *S. oliveri* in Diagram 4 shows greater growth in the 1 % solution than in the 3 %, as fewer vegetative branches were produced when flowers were formed.

The following conclusions may, I think, be deduced from these experiments:

1. *Salicornia oliveri*, Moss., and *Salicornia ramosissima* grow better in the presence of sodium chloride than they do in its absence, the greatest growth being when 2 % to 3 % of this salt is present. Higher percentages decrease the growth of the plants.

2. The effect of sodium chloride on the growth of *Suaeda maritima* is not so marked. Plants grow equally well in its absence as when a small quantity (1 %) is present. Growth decreased when a greater amount of salt is present, and decreases with the increase in amount.

3. *Salicornia ramosissima* and *Suaeda maritima* can resist the presence of a large amount of sodium chloride, as is seen from the 'pan' experiments

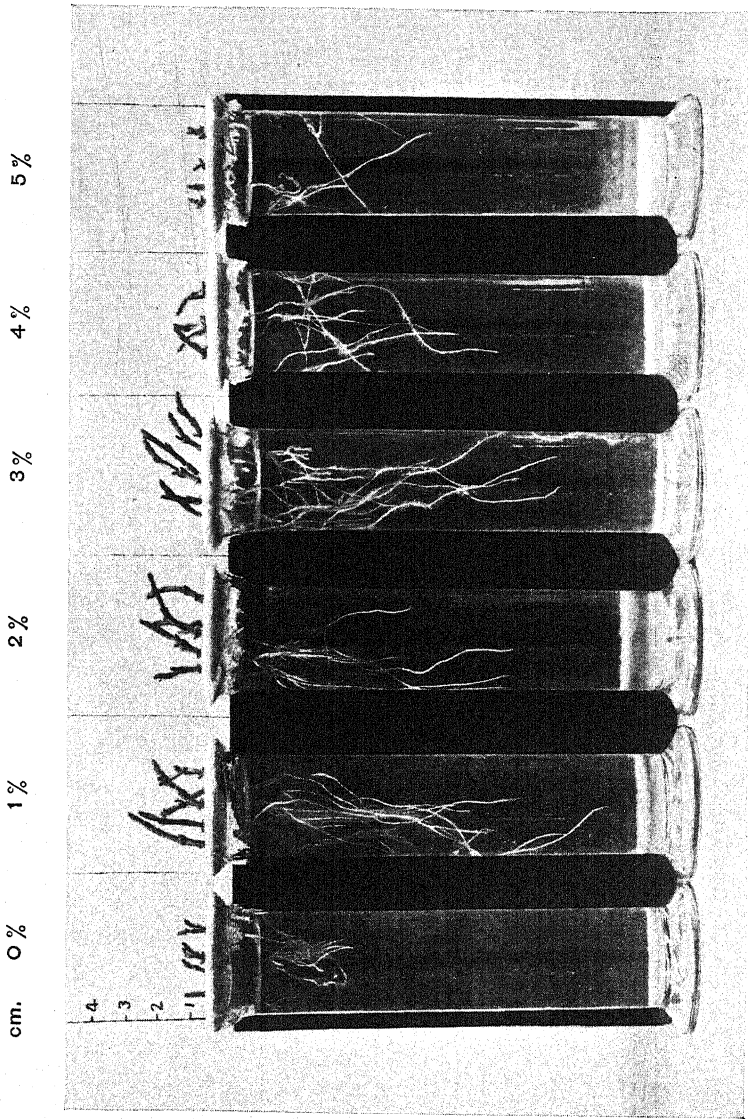
when the plants remained alive and green when the salinity of water in soil rose at times to 17%, though they were not able to grow.

4. The growth of *Glyceria maritima* is decreased with the increase of the salinity of the soil.

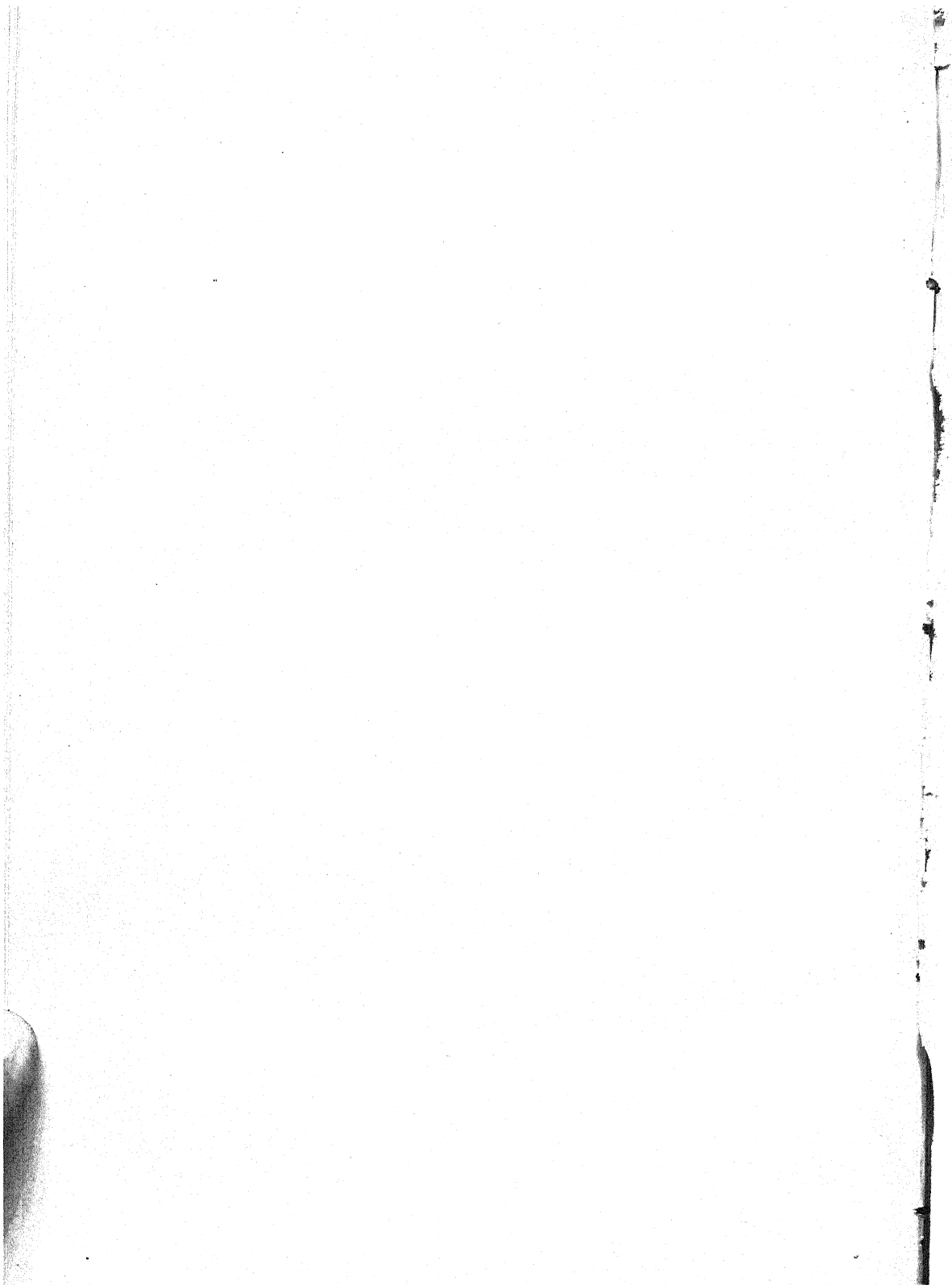
After these experiments had been made, my attention was drawn to a paper by Mr. J. A. Terras,<sup>1</sup> in which he records the results he obtained with experimental cultures of certain salt marsh plants. He grew plants of *Salicornia herbacea*, *Suaeda maritima*, *Glaux maritima*, *Plantago maritima*, and *Spergularia media* in sand in pots suspended in vessels containing nutritive solutions (Knop's) made up with varying proportions of filtered and sterilized sea water. He continued his experiments for the much longer period of six months, from April to September. The general results recorded are similar to those given above. The effect of the salt varied with the different plants. *Salicornia*, *Suaeda*, and *Glaux* grew best when sodium chloride was present, while the greatest growth of *Plantago* and *Spergularia* took place in the absence of this salt.

When the amount of growth in the varying concentrations of solution is compared, it is seen to be greatest in the case of *Salicornia* and *Suaeda* in those pots suspended in solutions containing 0.92% of sodium chloride; and the growth is found to decrease with increase in concentration of the solution. A difference is also seen on comparing the results of the higher percentages of salt, for plants of *Salicornia herbacea* and *Suaeda maritima* died in those pots in the liquid containing 3.2% of sodium chloride, while in the experiments described above these plants were able to grow in much greater percentages of salt.

<sup>1</sup> Terras, J. A.: Notes on the Salinity of the Cell-sap of Halophytes with Relation to that of the Soil Solution in which they grow. Proc. Scot. Micro. Soc., vol. ix, nos. ii and iii.



Series of water cultures of *Salicornia oliveri*, showing effect of various strengths of sodium chloride on growth.



# Note on Abnormal Flowers in *Orchis purpurea*, Huds.

BY

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With four Figures in the Text.

IN specimens of *Orchis purpurea*, Huds. gathered in Kent by one of the advanced students at Birkbeck College, several flowers were found to show various abnormalities in structure. A large number of inflorescences were examined, both in the field and in the laboratory, and out of three spikes sixteen flowers were obtained which presented deviations from the ordinary type of conformation.

The main abnormality consisted in an increase in the number of stamens. *Orchis purpurea*, like the majority of Orchids, develops normally only the anterior stamen of the outer whorl, A<sub>1</sub> (Fig. 1), while the other members, A<sub>2</sub>, A<sub>3</sub>, are represented by staminodes in the shape of auricles (Fig. 2). In some of the abnormal flowers the staminodes were replaced by stamens having both pollinia fully developed, so that the flower became triandrous (Fig. 3). In several cases only one staminode was transformed and diandrous flowers resulted, the stamens being either A<sub>1</sub>, A<sub>2</sub>, or A<sub>1</sub>, A<sub>3</sub>. In these diandrous forms the staminode which was not transformed maintained its ordinary position on the side of the column. This would seem to show that the staminodes are potentially stamens, and their appearance as such in a fertile condition goes *pari passu* with their disappearance as staminodes. This also means that the additional stamens are not derived from any other normally suppressed members of the androecial whorls. In one case a further departure from the type was observed. The stamens A<sub>1</sub>, A<sub>2</sub>, were fertile, and the posterior stamen of the inner whorl a<sub>3</sub>, which is usually completely suppressed, was represented by a pollinium arising

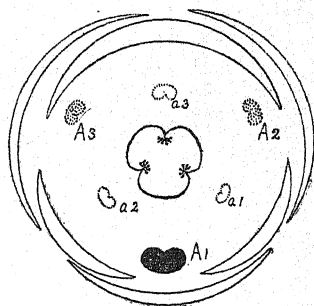


Fig. 1. Diagram of *Orchis* flower. The stamens A<sub>2</sub>, A<sub>3</sub>, are represented by staminodes on the side of the column.

from the mesochil. According to Pfitzer the odd stamen  $a_3$  does occasionally appear in abnormal flowers.

These abnormalities in the androecium were not always accompanied by any other malformation in flower structure. Two flowers showing the triandrous condition were otherwise quite normal (Fig. 3). On the other hand, cohesion of the sepals occurred in some, and in many flowers this was observed without, however, any modification in the androecial whorls. In the flower where  $a_3$  was represented by a pollinium the labellum was considerably modified. One lateral lobe was scarcely developed, and the middle lobe showed little trace of its usual bifid character.

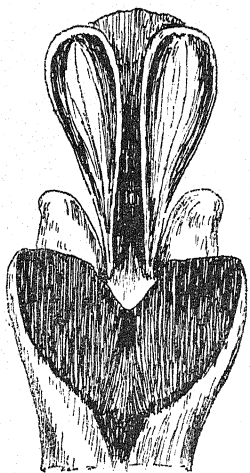


Fig. 2. Column from normal flower of *Orchis purpurea*, showing the staminodes.

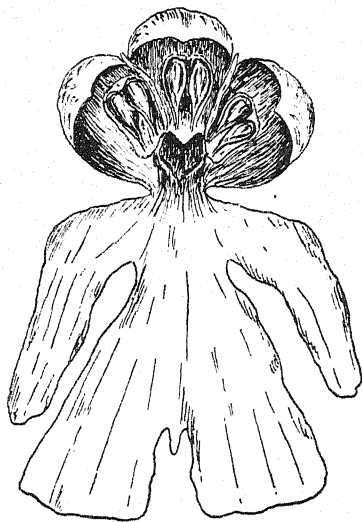


Fig. 3. Triandrous flower; the staminodes appear as fertile stamens.

Deviations from the typical structure in Orchid flowers have long been known. In 1825 Von Martius (1) recorded three anthers in *Orchis Morio*, and adds, 'monstrosa, anth. 3! singulae naturaliter conformata'. A hexandrous flower of *Orchis militaris*, to which *O. purpurea* is a close approach, has been described by Kirschleger (2). Further details of numerous abnormal forms are given by Masters (3), and more recently by Penzig (4). In many of these instances it appears that substitution occurred. The normally suppressed stamens appeared in a petaloid state. In my specimens the stamens were fertile, the tendency being towards a restoration of numerical symmetry.

It is not the purpose of this note to enter into a full discussion regarding the homologies of the Orchid flower. The discussion is well known, and has arisen because of the striking departure from the monocotylous type



which Orchids make, especially in the androecial and gynaecial whorls. A question that specially interested the earlier workers was whether all the stamens in a more or less complete state of development are confluent with the column, or whether the two lateral members of the outer whorl are incorporated with the labellum. Brown (5) advances the view that the small auricles of *Orchis* are essentially the same structures as the leaf-like staminodes in *Diuris*, and therefore form the complement A<sub>2</sub>, A<sub>3</sub>, of the outer whorl of stamens. But to this view Brown did not permanently hold. At first he believed the stamens A<sub>2</sub>, A<sub>3</sub>, were combined with the labellum. Cruger (6) contends that the labellum is a simple organ and not the union of one petal with two petaloid stamens. According to

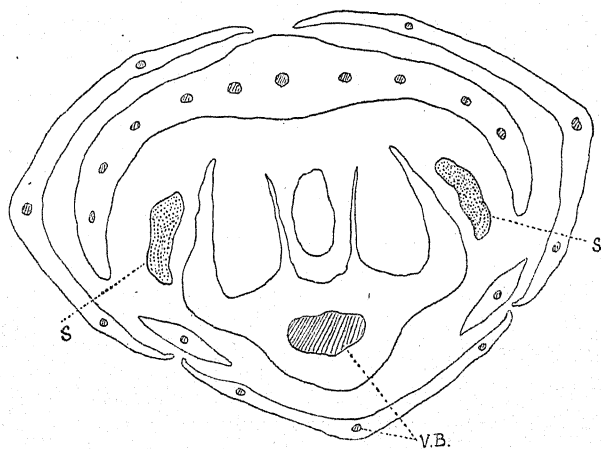


Fig. 4. Transverse section through bud of normal flower where the staminodes are free from the column. S. staminode; V.B. vascular bundle.

Darwin (7), whose views are based on the course of the vascular bundles in the flower, the labellum is a complex structure representing the fusion of the odd petal with the pair of outer stamens. This hypothesis has lost favour in view of the more recent study of floral development by Pfitzer (8). The number and distribution of the vascular bundles in the labellum are a physiological problem, and cannot always be accepted as morphological evidence. In *Orchis* Darwin found no trace of bundles in the auricles which he believed to represent the stamens of the inner whorl. Nor have I succeeded in tracing vessels to these structures in cases where the flower is normal. They are small and need no special system apart from the large bundle which supplies the column. But when they develop into stamens in such abnormal flowers as have been described, then I find that each is supplied with a vascular strand. As stamens their demand for nutrition is greater than when they remain as staminodes. The

distribution of bundles, in fact, would seem to be determined by physiological needs.

In the foregoing account I have referred to the auricles or staminodes as the representatives of the stamens A<sub>2</sub>, A<sub>3</sub>, for in a transverse section through the bud of a normal flower (Fig. 4) their relative position to the other floral parts indicates that they belong to the outer staminal whorl. I have already mentioned that in the diandrous flowers one of the staminodes remained normal. It is natural to assume, therefore, that in the triandrous condition the extra stamens represent both staminodes transformed, and, like the staminodes in a normal flower, belong to the outer androecial whorl. Thus it seems that the stamens A<sub>2</sub>, A<sub>3</sub>, are not associated with the labellum. This structure remains a simple organ whose analogue is to be found in various other families, and the explanation of its size and shape is to be sought for in biological connexions.

I wish to tender my thanks to Dr. H. C. I. Gwynne-Vaughan for kind help and criticism during the preparation of this note.

#### SUMMARY.

1. Diandrous and triandrous flowers of *Orchis purpurea*, Huds. found in Kent are described.
2. The extra stamens are the transformed staminodes.
3. The staminodes in a normal flower have no vessels, but when they develop as fertile stamens they are provided with a vascular supply.

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7. DARWIN, C. ('77): *Fertilisation of Orchids*.
8. PFITZER, E. ('89): *Orchidaceae*. Engler und Prantl, *Die natürlichen Pflanzenfamilien*, ii. Teil, 6. Abt., p. 52.

## NOTE.

**THE ANATOMY OF THE STAMENS IN CERTAIN INDIAN SPECIES OF PARNASSIA.**—The present note forms a supplement to a paper on the structure of the androecium in *Parnassia* which appeared in 1913 in the 'Annals of Botany'.<sup>1</sup> At that time the only members of the genus whose stamen-anatomy I had had the opportunity of examining were the European *Parnassia palustris*, L. and certain American species. However, since the paper was published, I have received through the kindness of Mr. G. H. Cave, Curator of the Lloyd Botanic Garden, Darjeeling, herbarium material of four species of *Parnassia* from the Himalayas—namely, *P. ovata*, Ledeb., *P. nubicola*, Wall., and *P. pusilla*, Wall., from a height of 14,000 feet, and *P. Wightiana*, Wall., collected at a height of 13,000 feet. I desire to express my gratitude to Mr. Cave and also to Mrs. Howard of Pusa, and Mr. C. C. Calder, Curator of the Herbarium, Royal Botanic Garden, Sibpur, who have rendered it possible for me to extend my study of the stamen-anatomy of *Parnassia* to the Indian species. The herbarium material received was treated according to the plan described in my previous paper.<sup>2</sup> It was again found that by this method serial sections, showing the detailed anatomy of the stamens, could be obtained from dried flowers.

*P. nubicola*, *P. Wightiana*, *P. ovata*, and *P. pusilla* all belong to the Section *Nectarotrilobos* of Drude,<sup>3</sup> whereas all the species studied in my previous paper were members of the Section *Nectarodroson*. It was thus of some interest to ascertain whether the Indian species showed the same anatomical peculiarities as those which I have recorded for *P. palustris* and its allies, or whether those peculiarities were confined to the Section *Nectarodroson*. The conclusion which I have reached is that, as regards the anatomy of the filament, these four Himalayan species closely recall those previously examined from other parts of the world. They present a series, which I regard as a reduction series, parallel with that which I have described in the American species *P. fimbriata*, Banks, *P. montanensis*, Fern. et Rydb., and *P. parviflora*, DC.<sup>4</sup>

*Parnassia nubicola*, which is a well-developed plant with relatively large flowers, shows features in the anatomy of the filament comparable with those observed in *P. fimbriata* and *P. palustris*, which it also resembles in size and habit. The xylem in the connective spreads and branches, while, in the filament below the attachment of the anther, the wood is roughly circular in transverse section, with protoxylem elements and sometimes a few thin-walled parenchyma cells occupying an internal

<sup>1</sup> Arber, A.: On the Structure of the Androecium in *Parnassia* and its Bearing on the Affinities of the Genus. Ann. Bot., vol. xxvii, 1913, p. 491.

<sup>2</sup> Arber, A.: l. c., p. 492.

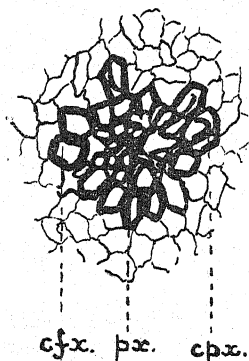
<sup>3</sup> Drude, O.: Ueber die Blüthengestaltung und die Verwandtschaftsverhältnisse des Genus *Parnassia*. Linnaea, Bd. xxxix, N. F., Bd. v, 1875, p. 314.

<sup>4</sup> Arber, A.: l. c., pp. 495-7.

position. In herbarium material, the phloem cannot be recognized with certainty. As regards the xylem, the bundle closely recalls certain forms assumed by the vascular structures in the filament of *P. palustris*, as may be seen by comparing the figure of the present note with Pl. XXXVI, Fig. 1 of my previous paper. (In both these figures the adaxial side of the stamen lies to the right of the diagram.)

In the next species, *P. Wightiana*, the bundle is small, and, though the xylem opens out in the connective, no other sign of complexity can be detected, except that there are occasional indications of a centrally-placed protoxylem in the filament. This Indian species has thus reached a stage of reduction in the stamen-anatomy comparable with that obtaining in the American form *P. montanensis*.

In *Parnassia ovata* and the minute species *P. pusilla*, the bundle is very small.



*Parnassia nubicola*, Wall.  
Transverse section of xylem group in filament a short distance below insertion of anther. The adaxial surface of the stamen would lie to the right of the diagram. *cfx.* = centrifugal xylem; *px.* = protoxylem; *cpx.* = centripetal xylem. ( $\times 636$ .)

I have found no trace here of the anatomical complexity in the filament, which seems to be confined to the larger members of the genus with stouter stamens. These two species may thus be compared with the American form *P. parviflora*, whose slender filaments are traversed by a simple bundle. It may be recalled that Hooker and Thomson<sup>1</sup> have suggested that *P. pusilla* and *P. ovata* may perhaps prove to be merely forms of *P. nubicola*. If this were so, it would confirm the view that their stamen-anatomy represents a stage in reduction from the more complex type found in *P. palustris*, *P. fimbriata*, and *P. nubicola*.

Since the presence of additional xylem elements on the adaxial side of the filament bundle has now been recorded in three species belonging to two different Sections of the genus *Parnassia*, and natives, respectively, of Europe, America, and Asia, and since, in the only cases so far examined in which this complexity is absent, the bundle may reasonably be regarded as having undergone reduction, we may infer that the structure in question was probably characteristic of the common progenitor of the genus. As I have previously suggested, the unique features observed in the anatomy of the androecium may be interpreted as indicating that the single stamen of *Parnassia* has been derived by reduction from an ancestral stamen-fascicle.

AGNES ARBER.

BALFOUR LABORATORY, CAMBRIDGE,  
June 5, 1914.

<sup>1</sup> Hooker, J. D., and Thomson, T.: Praecursores ad Floram Indicam. Journ. of Proc. Linn. Soc. Bot., vol. ii, 1858, p. 78.

# The Comparative Morphology of the Embryo and Seedling in the Gramineae.

BY

ETHEL SARGANT, F.L.S.

AND

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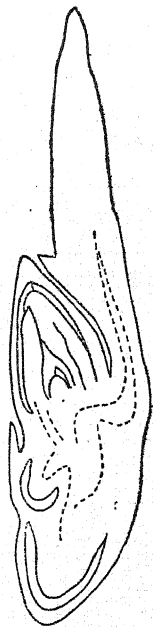
With Plates IX and X and thirty-five Figures in the Text.

THE embryo of the Grasses is sufficiently unlike that of most Monocotyledons to render exact comparison difficult. No question arises concerning the stem-bud and primary root; they are particularly clear in the Grass embryo, because it is more completely differentiated in the ripe seed than that of most Monocotyledons. But the parts which feed and protect them—the scutellum, coleoptile, coleorhiza, and epiblast—retain these non-committal names because botanists are not yet agreed on their respective homologies.

Before 1872 the only evidence considered was the structure of the embryo within the seed, or immediately on germination. At that age the vascular tissue is not sufficiently differentiated to be traced with certainty.

In 1872 Van Tieghem published a paper in which he compared the vascular skeletons of many seedling Gramineae with each other, and with those of certain other selected monocotyledonous seedlings. He interpreted the structure of the embryo within the seed in the light of its later development. In a subsequent paper (1897), the same author pursues the subject. Publishing no new figures, he accepts a correction of fact made by Miss Lewin ('87), Bruns ('92), and Schlickum ('96), but otherwise depends mainly on the evidence published in 1872. His interpretation of that evidence is, however, quite different.

Since that date the method then introduced by Van Tieghem has been applied to seedlings from many families, with a precision impossible before the introduction of the microtome. Indications of race



TEXT-FIG. 1.  
*Avena sativa*, L.  
Embryo in median  
section.  $\times 18$ .

history have been traced in the vascular system of seedlings belonging to other monocotyledonous families, to Dicotyledons, and to Gymnosperms.<sup>1</sup> The Grasses, however, have been neglected of late years, and we felt it desirable to repeat and extend Van Tieghem's observations on them with improved methods, and in the light of wider experience. This task we began together at Reigate in 1902, but owing to various interruptions it has only just been finished.

We wish to express our thanks here to Miss E. N. Thomas, D.Sc., for permission to use her preparations, notes, and drawings of the Zingiberaceae (see p. 209); to Mrs. G. R. Taylor for the loan of her preparations from *Triticum* and *Hordeum*; to Dr. O. Stapf, F.R.S., for various suggestions and criticisms; and to Mr. R. I. Lynch, M.A., of the Botanic Garden, Cambridge, for the gift of seeds and other material.

Besides the various types of Grasses, we have examined seedlings from other monocotyledonous families, choosing those in which the cotyledonary sheath is a prominent feature; and we have continued a detailed study of certain seedlings belonging to the Zingiberaceae which had been begun by Miss Thomas in the laboratory at Reigate. Her observations on the course of the bundles in the cotyledonary sheath of *Elettaria* suggested to us an explanation of the vascular skeleton in the coleoptile of *Avena*, which has been confirmed by further research. We think that the vascular symmetry of the seedling in this genus approaches that of the *Avena* type in the Gramineae, and gives a clue to the homologies of seedlings included in the other types.

Many of the conclusions which we draw from these studies are identical with those of Van Tieghem in 1872, of Schlickum in 1896, and others. We take a new view of the morphological nature of the mesocotyl, and this is perhaps the chief contribution which we make to the theory of the subject. The mass of evidence examined is considerable, and much of it is new. We divide it under two heads; first the description of certain seedling types among the Grasses themselves, and then that of a few selected species

<sup>1</sup> See Sargent, E., Presidential Address, Section K, Botany, Brit. Assn. Report, Birmingham, 1913. On pp. 703 and 704 will be found a list of references to papers dealing with the seedlings of Angiosperms and Gymnosperms from an anatomical standpoint. The following additional references may be added to this list:

Hill, T. G., and de Fraine, E.: A Consideration of the Facts relating to the Structure of Seedlings. *Ann. of Bot.*, vol. xxvii, pp. 257-72, four text-figs., 1913.

Hill, T. G., and de Fraine, E.: On the Classification of Seed-Leaves. *Ann. of Bot.*, vol. xxviii, pp. 359-62, 1914.

Lee, E.: Observations on the Seedling Anatomy of certain Sympetalae. II. Compositae. *Ann. of Bot.*, vol. xxviii, pp. 303-29, thirteen text-figs., 1914.

Mellor, A. E.: The Seedling Structure of *Dryas octopetala*. *The Naturalist*, 1911, pp. 310-12, six text-figs.

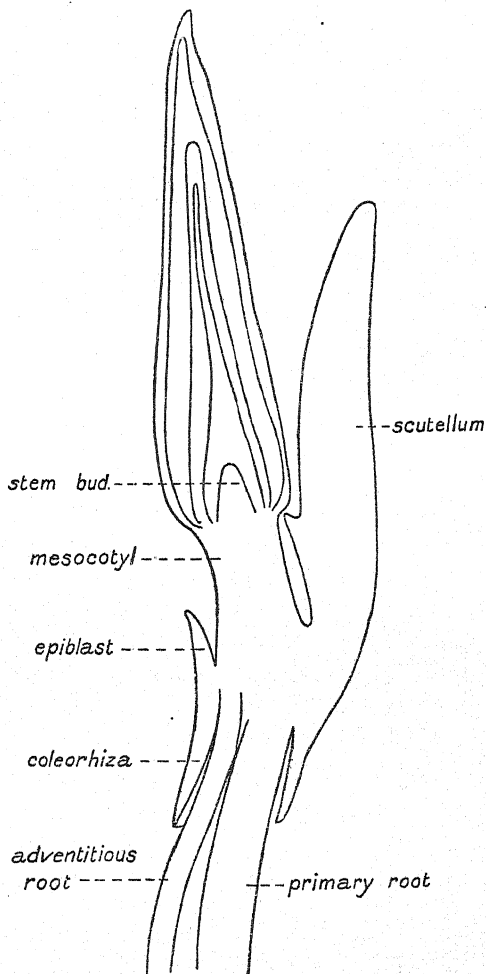
Thomas, E. N.: Seedling Anatomy of Ranales, Rhoeadales, and Rosales. *Ann. of Bot.*, vol. xxviii, pp. 695-733, two plates, forty-three text-figs., 1914.

from other Monocotyledons for comparison. But before describing our own researches, we will outline the points in dispute.

The members of doubtful homology are shown in the diagram of a seedling *Avena* (Text-fig. 2). The same parts are present in the embryo with the exception of the mesocotyl. This appears first on the elongation of the axis during germination (compare Text-figs. 1 and 2).

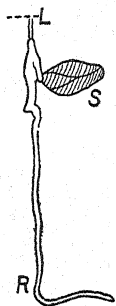
In the young seedling of a hypogeal Monocotyledon, two members are distinguished externally: the cotyledon, and the main descending axis. The plumular bud is present, but is commonly concealed within the expanded base of the cotyledon. This expanded base, with or without an appendage, forms the *sheath*, which protects the plumule during germination. The apex of the hypogeal cotyledon becomes the *sucker*, absorbing food from the endosperm. Sucker and sheath are usually connected by a *stalk*, which may be very short or absent. For instance in *Tigridia* (Text-fig. 8, p. 8) the seed appears to cling to the sheath, but on removal of the seed-coats a short neck is found between sheath and sucker.

The scutellum of *Avena* seems perfectly comparable with the sucker of *Tigridia* for example, and the coleoptile resembles such sheaths as those of *Tigridia*, *Crocus*, *Colchicum*, and *Elettaria*. But in *Avena* and similar forms, as in the *Zea* type also, scutellum and coleoptile are separated from each other by the mesocotyl, which is sometimes very long (Text-fig. 18, p. 179). If this were not so—that is, if throughout the Gramineae the coleoptile and scutellum were inserted on the axis at the same level (as in *Hordeum* and



TEXT-FIG. 2. *Avena sativa*, L. Diagram of part of very young seedling in median section.

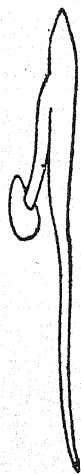
*Triticum*, for instance)—botanists would probably have found no difficulty in considering the scutellum as the sessile sucker of the cotyledon, and the coleoptile as its sheath. Van Tieghem, indeed, did take this view in 1872, and justified it by a bold morphological fiction. He treated the whole of the axial region separating the insertion of the scutellum from that of the coleoptile as an elongation of the first node. On that hypothesis it could not be considered as belonging either to epicotyl or hypocotyl, and Celakovský in 1897 (p. 145) embodied Van Tieghem's view in the term mesocotyl. We are using this term because it is convenient and generally understood, but we do not accept the hypothesis which gave rise to it.



TEXT-FIG. 3.  
*Iris pseudacorus*,  
L. Seedling, life-size.

Our own view of the nature of the mesocotyl is founded on the comparative study of the vascular skeleton in Grass seedlings and in other Monocotyledons. The facts on which it is based will be described in detail later. We think, however, that they will be followed with greater ease if we outline our hypothesis at once.

The cotyledonary sheath of Monocotyledons assumes many forms. The simplest case is that in which it is nothing more than the expanded base of the cotyledon wrapped round the plumular bud. This is very common in epigeal species, as in *Allium*. In hypogeal seedlings the expanded base is commonly transformed into a closed cylinder (*Arum maculatum*, L., *Veratrum nigrum*, L., &c. Another variant on this type is illustrated by *Iris pseudacorus*, L. (Text-fig. 3), and *Commelina coelestis*, Willd. (Text-fig. 35, p. 217), where the stalk of the cotyledon is bent sharply downwards just where it begins to expand into a sheath. The effect is sometimes to form a sort of hood over the young plumule.



TEXT-FIG. 4.  
Diagram of imaginary seedling, to illustrate how the stalk of the cotyledon might fuse with the hypocotyl.

But in other cases when the stalk is bent downwards in this way, the hood is formed by an appendage to the expanded base (*Tigridia*, Text-fig. 8, p. 168, and *Elettaria*, Text-fig. 30, I, p. 210). Such a hood often suggests a pair of stipules united along one margin or both. Van Tieghem ('72, p. 271) distinguished this structure as the upper or stipular sheath from the lower or basal sheath. Occasionally the latter is almost wholly suppressed, while the upper sheath is well developed (*Kniphofia*).

Now suppose a seedling with a pronounced upper sheath to its cotyledon, and no basal sheath at all, the stalk of the cotyledon pointing downwards and in close contact with the axis (Text-fig. 4). If we imagine



stalk and axis to become completely united, the sucker will appear sessile on the axis at one level, while the sheath is inserted higher up. These are precisely the relative positions of scutellum and coleoptile in *Avena*. As far as external structure goes, the mesocotyl may have been derived in some such way from a fusion of cotyledonary stalk with hypocotyl. What influence would such an ancestry be likely to have on the internal anatomy?

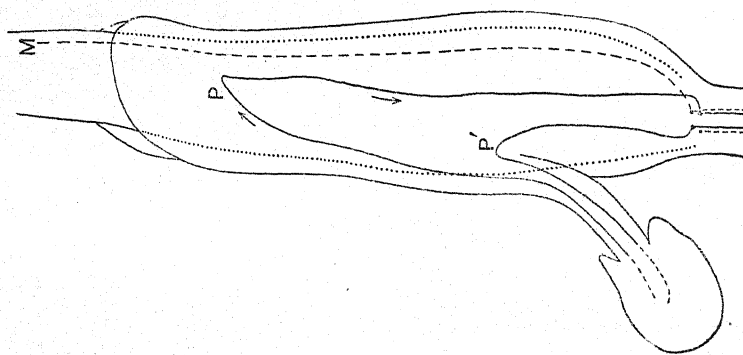
In such an ancestor the stalk, before it became fused with the axis, would contain at least one bundle; and every bundle present would travel upwards within the stalk to the first node, where the sheath is also inserted. At the first node each stalk-bundle would enter the axis. After fusion of stalk and axis all the stalk-bundles would be enclosed within the same compound structure as the stele of the axis. They would to all appearance be traces lying side by side with the stele during this part of its course, and on reaching the first node they would enter either the sheath or the stele. We have compared this imaginary skeleton with that of *Avena*, or rather with that of the *Avena* type, which we consider the primitive form of Grass seedling.

The *Avena* skeleton is complicated, but its main features can perhaps be followed with the aid of a diagram (Text-fig. 6, p. 166). The scutellum is sessile, and its single massive bundle turns upwards on entering the axis without joining the stele. Transverse sections through the mesocotyl show the scutellum trace outside the stele, and inverted with respect to it (Pl. IX, Fig. 4). The inverted trace is clearly double, having two groups of soft bast, and in older seedlings two formations of metaxylem also, with a single group of protoxylem elements. Here, then, is a trace outside the stele of the mesocotyl, and travelling from scutellum to first node; just as in our imaginary case a trace or traces travelled from sucker to first node side by side with the stele of the hypocotyl.

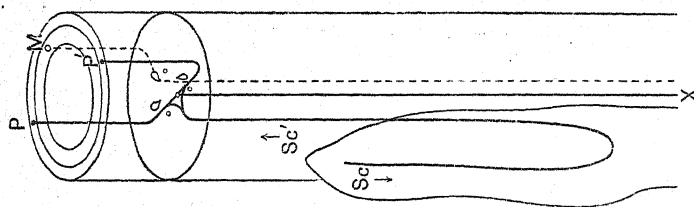
Reference to Text-fig. 6 shows that the scutellum trace divides into two parts at the first node while still outside the stele. The phloem group on either side becomes a phloem group of the double coleoptile trace on that side, and half the xylem goes with it. Thus the scutellum trace ceases to exist at the first node, but each of its two halves maintains its identity within one of the two coleoptile traces. The other half of each coleoptile trace is supplied from the stele.

The peculiar connexion between scutellum trace and coleoptile traces can be equally well described from above downwards; that is, by following the coleoptile traces through the first node.

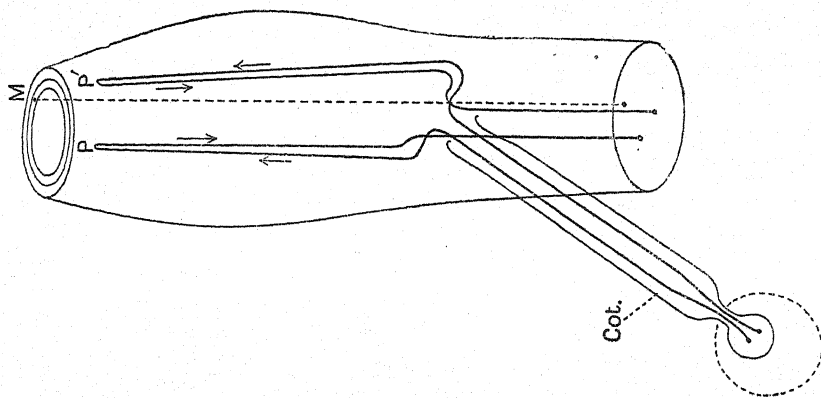
The structure of the first node is symmetrical about a plane which bisects the first leaf, and when prolonged downwards also bisects the scutellum trace and the scutellum itself. It is the plane of the paper in Text-fig. 2. In Text-figs. 16 and 17, pp. 174 and 175, it is represented by the shorter axis of the elliptical diagram, which always bisects both *sc.*, the scutellum trace,



TEXT-FIG. 5. Diagram of *Ellettaria* sheath, much enlarged, showing vascular skeleton.



TEXT-FIG. 6. Diagram showing the vascular skeleton of scutellum, mesocotyl, and coleoptile (base only) in seedling of *Arenaria* type.



TEXT-FIG. 7. Diagram of vascular skeleton in imaginary type X.

and also *M*, the midrib trace from the first leaf. The coleoptile bundles are bisected by the long axis at right angles to this. They enter the stele at opposite sides, and the adjacent plumular traces are inserted on them. Then each coleoptile trace divides. The phloem groups separate, one turning outwards and carrying some xylem elements with it, while the other continues within the stele, and meets the corresponding branch from the opposite coleoptile trace there. These united branches turn downwards, and constitute the bundle *x* within the stele (Text-figs. 6, p. 166, and 16, p. 174). The outward branches meet too, but outside the stele. Together they build up the scutellum trace.<sup>1</sup>

The direct connexion between scutellum trace and coleoptile bundles is the most striking feature in the *Avena* type, and we have therefore searched for some special relation between sucker and sheath in the vascular structure of other monocotyledonous seedlings. The comparison which we think most instructive is with the seedling of *Elettaria cardamomum*, one of the Zingiberaceae. In this species the sucker of the cotyledon remains within the seed, and is connected with the axis by a fairly long stalk. The sheath is partly above the insertion of the stalk, and partly below it. The stalk runs up to its insertion, and in that neighbourhood is commonly almost parallel with sheath and axis (Text-fig. 5). The two bundles of the cotyledon are symmetrically placed within the stalk, the xylem of each being internal. On entering the sheath, however, they diverge to right and left. One travels upwards, and approaches the apex of the sheath before turning sharply down. The other turns down almost as soon as it enters the sheath (Text-fig. 5). In the end they enter the stele of the hypocotyl from opposite sides. The traces which separate them are plumular.

We have constructed an imaginary vascular skeleton to link the symmetry of the *Avena* type with that of *Elettaria* (Text-fig. 7). The stalk of the cotyledon contains two independent bundles. These enter the axis at the first node, where each turns outwards into the cotyledonary sheath, and then upwards within it. Near the apex each turns sharply

<sup>1</sup> The vascular skeleton described above is that of the *Avena* type, generalized from *Avena* and from other genera resembling it in seedling structure, notably from *Zizania*. It differs in one important respect from the vascular skeleton of *Avena* itself. In describing the latter we shall show later on that the scutellum trace is directly connected with the stele by a massive xylem arch (p. 178). We have come to the conclusion that this feature is adaptive, not primitive; perhaps a device for supplying the scutellum with water from the lower roots in the most direct way. For in *Zizania*, although, as usual in an aquatic species, the whole xylem skeleton is very much reduced, it is still identical with that of *Avena* except in this one respect. No general reduction of xylem elements would account for the complete disappearance of a massive xylem arch between scutellum and stele. But the submerged scutellum of *Zizania* needs no short cut to secure an adequate supply of water, and this species has no lower system of cauline roots.

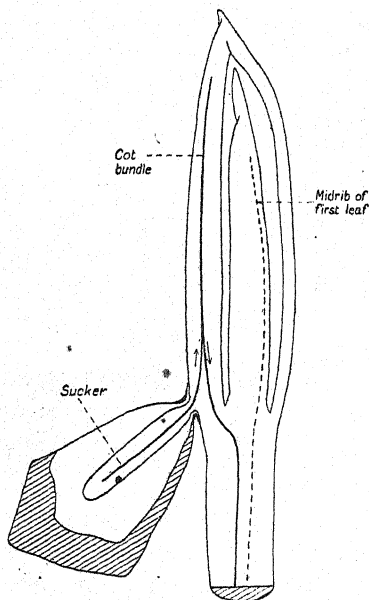
There are some independent grounds for considering *Zizania* as a genus with primitive features, particularly its floral structure.

downwards, doubling on itself in such a way that in transverse section two double bundles appear on opposite sides of the sheath. When the downward bundles approach the first node they leave their companions and enter the stele among the plumular traces.

This imaginary type *X* resembles *Elettaria* in possessing two distinct bundles in the cotyledon, which make an acute angle in the sheath before entering the stele of the hypocotyl from opposite sides. To derive it from *Elettaria* the stalk of the cotyledon must be inserted on the axis rather than on the sheath; both bundles must approach the top of the sheath before they turn down, and they must double on themselves so closely that

the upward and downward segments of each bundle are in contact throughout the upper part of the sheath.

For such close doubling of a bundle there is a precedent in the seedling of *Tigridia* (Text-fig. 8, p. 168). A single bundle enters the sheath from the cotyledon, travels up the dorsal spine of the sheath, and doubles on itself near the top. It appears in transverse section as a double bundle with two external xylem groups, dorsal and ventral, for some distance below the turn (Pl. X, Fig. 15). This unusual orientation of xylem and phloem depends on the median position of the bent bundle in the *Tigridia* sheath, and would not occur in the lateral bundles of the *X* sheath, however closely each might bend on itself.



TEXT-FIG. 8. Diagram of bundles in sucker and sheath of *Tigridia*.

To derive the vascular skeleton of the *Avena* type from that of *X*, four modifications are necessary. (1) The two bundles of the cotyledon must unite with each other throughout their course in sucker and stalk. Examples of such union are not uncommon among Monocotyledons.<sup>1</sup> The distinct bundles of one species may be represented by a double bundle in another species within the same genus. (2) The stalk of the cotyledon must unite with the hypocotyl so completely that the sucker appears sessile on the axis at a level below that of the first node. Thus a mesocotyl is formed by fusion of the cotyledonary stalk with part of the hypocotyl.

<sup>1</sup> Sargent, E. ('03): A Theory of the Origin of Monocotyledons founded on the Structure of their Seedlings. Ann. of Bot., vol. xvii, pp. 20-1, 1903. *Scilla sibirica* has two separate bundles in the cotyledon, and *S. peruviana* a double bundle. Cf. also the case of *Colchicum autumnale* mentioned below, p. 218.

The effect of these two structural alterations is that the stalk bundles are represented in the mesocotyl by a trace, distinct from the stele and parallel with it. The origin of this trace in *Avena* is suggested by its double structure and inverted orientation.

(3) In the sheath the upward and downward segments of each lateral bundle must be united to the very base. (4) The downward segments on entering the stele must fuse with each other to form trace  $x$  (IV in Text-fig. 16, p. 174).

These four modifications are sufficient to transform the imaginary type  $X$  into a vascular skeleton, with all the features which we consider as essential to the *Avena* type. Three more would be necessary to complete the resemblance to the actual *Avena* seedling. The sucker of  $X$  must become the scutellum of *Avena*; the sheath-bundles must be capped with xylem elements; and a xylem arch must be thrown from the scutellum trace to the mesocotylar stele.

All these characters are in our opinion adaptive, and therefore of secondary importance for our present purpose. The greater differentiation of the scutellum, as compared with the sucker, is closely related to the rapid growth of the Grass seedling in comparison with that of other Monocotyledons. The xylem caps to the coleoptile bundles are doubtless organs for the excretion of water. We have already referred to the probable function of the xylem arch as a water-carrier.

Our interpretation of the doubtful members of the *Avena* seedling is thus as follows:

The *scutellum* is the sucking apex of the cotyledon.

The *coleoptile* is the cotyledonary sheath—perhaps equivalent to a pair of stipules.

The *mesocotyl* is the fusion of the cotyledonary stalk with the hypocotyl.

The *coleorrhiza* and the *epiblast* we consider as outgrowths from cotyledon or axis, or both, and of little morphological importance.

The reconstruction of the missing link  $X$ , through which the Grass embryo and seedling are connected with those of hypogeal Monocotyledons in general, is intended to demonstrate how the following exceptional features of the *Avena* seedling may have arisen: the double scutellum trace and its inversion; its indirect connexion with the bundles of the stele through the coleoptile traces; the double structure of the coleoptile bundles. Later on, the reconstruction of  $X$  will be shown to throw light on the *Zea* type also, with its more specialized mesocotyl.

## I. COMPARATIVE ANATOMY OF GRASS SEEDLINGS.

A. *Avena* type.

*Avena sativa*, L. The structure of the embryo within the seed is well known. Only such details as are important for our purpose need be described here. A median section is outlined in Text-fig. 1, p. 161.

The scutellum is fleshy, but leaf-like in outline. It has a well-developed dorsal ridge (Text-fig. 13), and a ventral scale (Text-fig. 14). Whatever the morphological nature of this scale may be, its function is quite clear. The endosperm—which encloses the upper part of the scutellum like a cap—fits into the furrow between scutellum and scale, and ends there. Thus the plumule, which lies immediately below the scale, is quite clear of the endosperm, and is not impeded by it when growth begins. The scale is at some

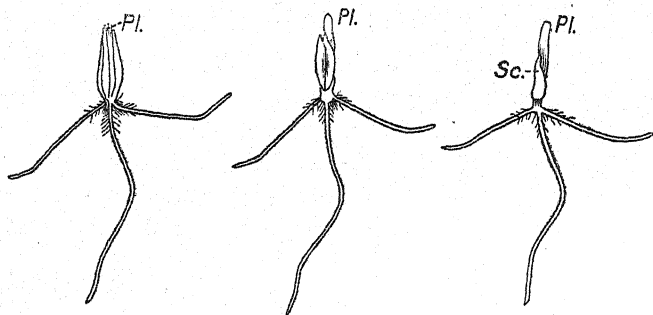


Fig. 9.

Fig. 10.

Fig. 11.

TEXT-FIGS. 9-11. *Avena sativa*, L. 9. Whole seedling, life-size. 10. Same seedling with husks of grain removed. 11. Same seedling with scutellum laid bare.

distance above the insertion of the scutellum on the axis, and the scutellum itself is prolonged below its insertion.

The first region of the axis to elongate is the mesocotyl, but the primary root is the first member to show externally in the germinating grain. In Text-fig. 9, the primary root is an inch and a half long, while the tip of the stem-bud can hardly be perceived among the glumes. Two cauline roots from the zone of insertion are also well developed.

The mesocotyl bears the stem-bud enclosed in the coleoptile, a stiff conical sheath with a sharp point. In terrestrial Grasses the coleoptile serves to force a way upwards through the soil, while protecting the stem-bud within it.

The mesocotyl may continue to grow for a considerable time. When grains are scattered on the ground in the usual way, the ascending axis of each grows upwards as soon as it can get clear of the glumes, making an angle of about 90° with the long axis of the grain. The length of the mesocotyl is then very variable. Growth appears to cease when the base

of the plumule is in a position favourable for the cauline roots it throws out. In a seedling produced from a grain which had been planted with the glumes pointing upwards, the mesocotyl ceased to grow when it had just overtopped them. Two foliage leaves were expanded at that time, and a third was appearing from the sheath of the second. The upper limit of the mesocotyl was clearly defined in this seedling by the sudden increase in diameter of the epicotyledonary axis with its sheathing leaves, and also by the appearance of rudimentary cauline roots at the first node (Text-fig. 15). By this time the food reserves of the endosperm are exhausted

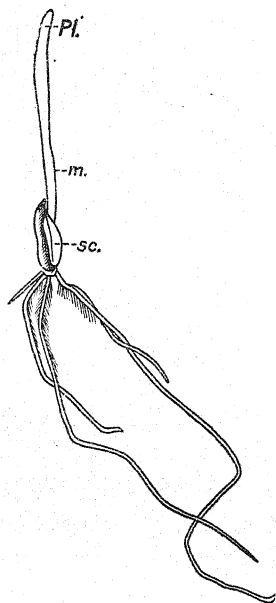


Fig. 12.

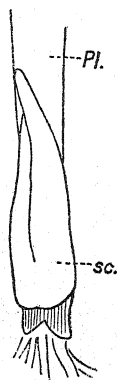


Fig. 13.

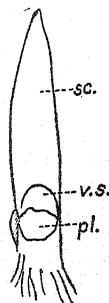


Fig. 14.

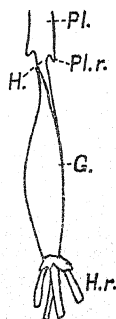


Fig. 15.

TEXT-FIGS. 12-14. *Avena sativa*, L. 12. Whole seedling, life-size. Part of grain is removed, exposing half scutellum. 13. All grain removed. Scutellum and adjacent parts from back.  $\times 4$ . 14. Scutellum from front, showing ventral scale (*v.s.*). The scar *pl.* shows place whence plumule has been removed.  $\times 4$ .

TEXT-FIG. 15. *Avena sativa*, L. From much older seedling, which showed three leaves. Grain with median region of axis attached. The mesocotyl is surrounded by the husks of the grain. Nodal roots show as rudiments.  $\times 3$ .

and the scutellum is functionless. The young plant will depend in the future on its green leaves only for supplies of food, and these leaves are developing a root-system of their own. The older root-system perishes by degrees; the cauline roots first, and then the primary root. In time all the lower members of the seedling—scutellum, mesocotyl, primary and insertion roots—having served their purpose, will disappear and leave the mature plant rooted at the first node.

We are concerned here, however, with the structure of the seedling in its early stages, before the epicotyledonary members have become functional.

At this period—that is, up to the time when the foliage leaves burst through the coleoptile—the seedling may be considered as an expanded embryo. Its ascending axis is the mesocotyl, crowned by coleoptile and stem-bud. The scutellum is inserted at the base of the mesocotyl, just where it passes into the primary root or descending axis. From the zone of this insertion spring several cauline roots (Text-figs. 9, 10, 11).

In a seedling of this age the plumule must depend on the primary root-system until it has developed its own roots. The first two leaves draw their water at first from the lower roots through the mesocotyl into which their traces are prolonged. Similarly, they draw their proteid material from the endosperm through the scutellum. At this period there is a great demand for proteids in the neighbourhood of the first node, for new members are constantly being formed there—the roots of the nodal system, rudimentary leaves at the growing point, and buds in the axils of the leaves. Hence no doubt the necessity for connecting the plumular traces directly with the vascular system of the scutellum. Later on the green leaves will furnish a supply of proteids to this region by their own activity.

The scutellum contains a single massive bundle, which follows the dorsal ridge and is equidistant from either surface. It is circular or oval in transverse section near the base, but towards the apex it spreads out into a crescent which repeats the convex outline of the dorsal epithelium. Occasional short branchlets are given off from the lateral wings, but they are not numerous, and they terminate at some distance from the epithelium. The carrying system is very well developed in this region of the scutellum, where the epithelium fringes both surfaces. On the whole, however, the outlying tissue is reached rather by lateral extension of the main bundle than by branching.

The lignified elements of the extended bundle are scattered. They are most numerous in the median zone, where they often appear as a band of lignified tissue with protoxylem elements at either extremity. The bulk of the phloem fringes this band on the dorsal or convex edge. But groups of elements with thick contents, which probably represent phloem too, are found among scattered lignified elements on the ventral side of the xylem band. Lower down, where the bundle is oval or circular in outline, the xylem elements are collected on the ventral side of it, the phloem on the dorsal.

At the apparent insertion of the scutellum, its bundle turns sharply upwards within the axis, but does not enter the stele (Text-fig. 6). Transverse sections through the mesocotyl show the slender stele in the centre, and on one side of it a massive inverted bundle—the scutellum trace (Text-fig. 16, p. 174, and Pl. IX, Fig. 5). They run upwards side by side to the real insertion of the scutellum at the first node.

Throughout its upward course in the axis, the scutellum trace is clearly



double (Pl. IX, Fig. 5). In seedlings of the age drawn in Text-fig. 12, p. 171, the two groups of soft bast can be distinguished very clearly by their contents. At this period the vessels of the phloem are crammed with proteids, which they are conveying from the endosperm to the first node. The two groups of soft bast are injected as it were with thick contents, which take up most stains readily, and then appear in transverse sections as two highly coloured patches within the trace (Pl. IX, Fig. 1). They are separated from each other by a partial sheath of bast fibres, and by the xylem group. In serial sections through the mesocotyl of older seedlings the bast vessels of the scutellum trace are nearly empty. The two phloem groups, however, are still defined by the greater differentiation of the bast fibres which partially sheathe them. The xylem also is better lignified, and shows two metaxylem groups with a common cluster of protoxylem elements between them (Pl. IX, Fig. 2).

In short, the structure of the scutellum trace is undoubtedly double as it approaches the first node. Here it will meet traces from the coleoptile and plumule as they enter the stele of the mesocotyl. From this region also the plumular or nodal roots will be given off later, and their rudiments are present even in the youngest seedlings we have cut.

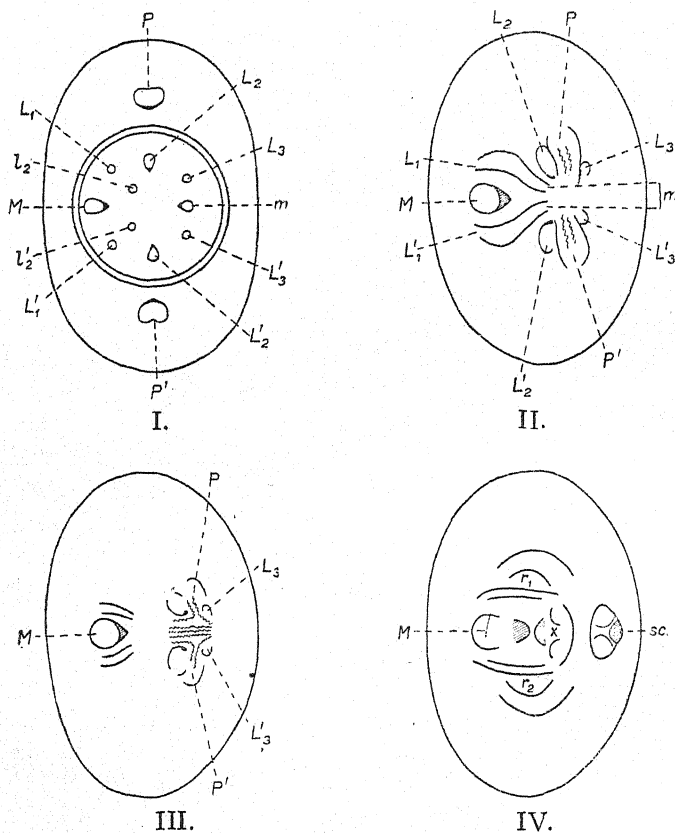
The vascular complex at the first node is best attacked from above. The stele of the mesocotyl is built up of traces from the plumule and coleoptile; these must therefore be followed downwards until they are joined by the scutellum trace. Considerable disturbance is caused at and near the node, by the insertion of cauline roots.

A transverse section taken just above the first node shows twelve traces arranged as in Diagram I, Text-fig. 16, p. 174. Seven of the ten plumular traces ( $M$ ;  $L_1$ ,  $L_2$ ,  $L_3$ ;  $L'_1$ ,  $L'_2$ ,  $L'_3$ ) belong to the first leaf; three ( $m$ ,  $l_2$ ,  $l'_2$ ) to the second. They are arranged in two concentric circles.  $M$  and  $m$  are midrib traces, and they face each other on the same diameter. The coleoptile bundles  $P$  and  $P'$  are bisected by a line perpendicular to this diameter.

The midrib trace  $M$  from the first leaf passes through the node unchanged. No other trace is inserted on it, nor does it contribute vascular elements to any cauline root. The midrib trace  $m$  from the second leaf behaves differently. It divides into two branches, one of which unites with the lateral traces  $l_2$  and  $L_1$ , the other with  $l'_2$  and  $L'_1$  (Diagram II, Text-fig. 16, p. 174). This occurs at the very top of the node, just as the coleoptile traces  $P$  and  $P'$  run into the stele. The gap left by  $m$  is clear in several consecutive sections below this level.

The union of  $L_1$  with half of the three principal traces from the second leaf gives rise to a lateral plate of internal xylem and external phloem. A similar plate is formed on the other side of  $M$  by the union of  $L'_1$  with the other second-leaf traces. The steles of the two first-formed nodal roots

will be inserted on these two plates respectively. But in such seedlings as those drawn in Text-figs. 9 (p. 170) and 12 (p. 171), where the stem-bud is still enclosed in the coleoptile, these roots are mere rudiments in which the vascular tissue is not differentiated, and the lateral plates are so far embryonic too, that xylem cannot be clearly distinguished from phloem. Their presence, however, affects the mesocotylar stele even at this age, and

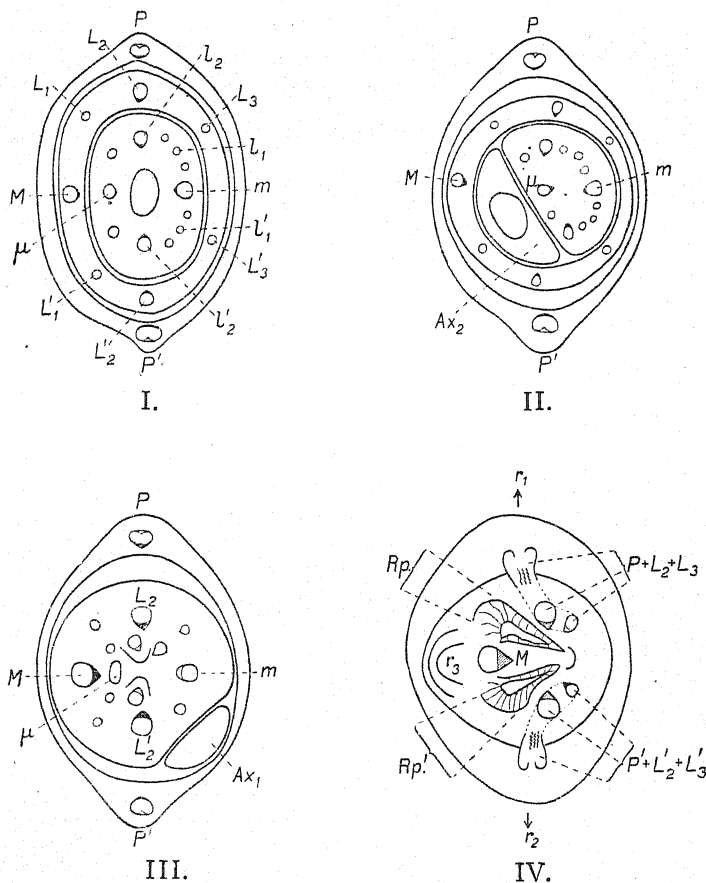


TEXT-FIG. 16. *Avena sativa*, L. Structure of axis in seedling about age of that drawn in Text-fig. 12, p. 171. Shown in a series of diagrams representing transverse sections from above downwards. I. Plumule surrounded by coleoptile. II. Top of first node. III. Base of first node. IV. Mesocotyl.

they are later of great functional importance. The anatomy of the young node is obscure until the structure of these plates is thoroughly understood, and it is therefore worth while to describe them in an older seedling where they are better differentiated.

In the oldest seedling examined, the third foliage leaf is just unfolding, and the second internode of the plumule is 2 mm. long. It is enclosed within the sheath of the first leaf, which is itself surrounded by the base of

the coleoptile. The internode contains a circle of twelve traces (Diagram I, Text-fig. 17): seven derived from the second leaf ( $m$ ;  $l_1, l_2, l_3$ ;  $l'_1, l'_2, l'_3$ ), and five from the third. The midrib of the third leaf is marked  $\mu$  in Diagram I. At the second node seven traces from the first leaf enter the axis, and form an outer circle (Diagram III, Text-fig. 17).



TEXT-FIG. 17. *Avena sativa*, L. Diagrams from much older seedling, showing base of plumule. I. Second internode. Axis sheathed by base of first leaf as well as coleoptile. II. Approaching second node.  $Ax_2$  is bud in axil of first leaf. III. Base of first internode. Axis sheathed by coleoptile only.  $Ax_1$ , bud in axil of coleoptile. IV. First node.  $Rp, Rp'$ , root-plates.

The first internode is undeveloped: there is no interval between the second and the first node. The upper limits of the two nodes are, however, marked by the insertion of two lateral buds,  $Ax_2$  in the axil of the first leaf (Diagram II), and  $Ax_1$  in that of the coleoptile (Diagram III). Both buds are embryonic, and do not yet receive any vascular elements from the stele.

Thus nineteen plumular traces enter the second node in a seedling of this age, as against ten in the younger one. Without following the course of the nine new bundles in detail, we may briefly say that they appear to fuse with one or other of the root-insertion plates. The midrib trace  $\mu$  divides itself between them. The total effect is to add a massive layer of external xylem to both plates.

Each now consists of two xylem layers with a thin sheet of phloem sandwiched in between them. Elements of large lumen are characteristic of the xylem. Their thickened walls are very well lignified, and they give a distinct character to the root-plates (Pl. IX, Fig. 5).

Three nodal roots are commonly inserted on the two root-plates. The latest formed ( $r_3$  in Diagram IV, Text-fig. 17) is also on the highest level. It appears behind  $M$ . In the seedling from which the diagrams of Text-fig. 17 (p. 175) were taken, this root was still rudimentary, but xylem elements from both plates are already moving round to the back of  $M$  to provide for its insertion. In older seedlings the plates will clearly meet behind  $M$ , almost enclosing it in a deep crescent or horseshoe.

Two older roots,  $r_1$  and  $r_2$ , are inserted lower down. One is attached to either plate, and their position is indicated by arrows in Diagram IV.

The root-plates are continued down the mesocotyl in a rather simplified form. Below the disturbance caused by root-insertions, the xylem layers become single again, and appear in transverse section as two lateral chains of five to six large thick-walled elements with groups of smaller ones among them. Pl. IX, Fig. 4 is drawn from a section not far below the first node, but the stele has almost assumed the appearance which it will maintain throughout the mesocotyl.

The mesocotyl ends with the insertion of three cauline roots, occupying the same positions with regard to the stele as the nodal roots at the top of the mesocotyl. One is ventral—that is, given off from behind  $M$ —and two are lateral—external to the two root-plates. Their steles are all inserted on the root-plates, which thus serve to connect the two root-systems of the seedling. Below this level the mesocotylar stele is prolonged into that of the primary root, but the method of transition from stem to root structure is almost completely masked by the disturbance caused by root-insertions. The root-plates end with the mesocotyl. Some of their larger elements, particularly those on the scutellum or dorsal side of the stele, can be followed into the stele of the primary root.

Before leaving the subject of the root-plates, we may observe that they are built up at the node from the main traces of the second and third leaves, which are thus put into connexion with the nodal roots. But only two minor traces from the first leaf enter the root-plates; the main lateral traces, as well as the midrib, are independent of them, and connect the first leaf with the primary root and the rest of the lower root-system.

Returning to the first node of the younger seedling, the midrib trace *m* from the second leaf has only just divided itself between the embryonic root-plates when the coleoptile traces *P* and *P'* run in from either side. As they do so, traces  $L_2$  and  $L_3$  are inserted on *P*, and  $L'_2$ ,  $L'_3$  on *P'*, in a way which must be described in detail.

The double structure of *P* and *P'* is clearly indicated, particularly in the neighbourhood of the node. Each has two distinct groups of soft bast (Pl. IX, Fig. 1). In young seedlings the common group of protoxylem only is lignified, but in older ones the metaxylem forms two distinct groups (Pl. IX, Fig. 2), and the protoxylem is often torn away, leaving a gap.

As the coleoptile traces enter the stele, the two phloem groups of each are separated by the xylem. One phloem group from *P* unites with that of  $L_2$ , the other with that of  $L_3$ . Similarly the two phloem groups of *P'* unite with those of  $L'_2$  and  $L'_3$  respectively (Diagram II, Text-fig. 16, p. 174).

The xylem of *P* divides. One branch turns inwards, that is towards the centre of the stele, and carries the xylem of  $L_2$  with it: the other—turning outwards—carries off the xylem of  $L_3$ . The xylem of *P'* behaves similarly, the inward branch sweeping off with the xylem of  $L'_2$ , and the outward one with that of  $L'_3$  (Pl. IX, Fig. 3).

In the end the ingoing xylem branches unite to form the xylem group  $x_2$ , which thereafter occupies the gap opposite *M*, and becomes part of the mesocotylar stele. The corresponding phloem groups accompany the xylem, remaining external to it (Diagram IV, Text-fig. 16, p. 174). For some distance below the node the two phloem groups are seen in favourable preparations to be distinct, but before the transitional region is reached they commonly form a single group. Both xylem and phloem can be distinguished in good preparations from other tissues of the mesocotyl throughout its length.

The outgoing branches of xylem from *P* and *P'* leave the stele altogether, and are followed by the phloem groups ( $\frac{1}{2} P + L_3$ ) and ( $\frac{1}{2} P' + L'_3$ ) respectively. United they form a double trace running downwards side by side with the central stele (Diagram IV, Text-fig. 16, p. 174, and Pl. IX, Figs. 4 and 5). The two phloem groups remain distinct within the trace until it approaches the scutellum. There are two groups of metaxylem, divided more or less completely from each other. The common group of protoxylem is directed towards the periphery of the mesocotyl (Pl. IX, Figs. 4 and 5).

The trace thus formed loses its double character as it reaches the insertion level of the scutellum, and turns sharply upwards to enter it (Text-fig. 2, p. 163).

This account of the course of the bundles in the first node must be understood to give the ground-plan of its structure as observed in seedlings of different ages. In such a tangle of traces the junctions are not accomplished with mathematical precision. For example, metaxylem elements

may enter the lateral root-plates from the plumular traces  $L_2$  and  $L'_2$ , though the bulk of their xylem—including all the protoxylem—unites with the ingoing branches of  $P$  and  $P'$  respectively. A more constant and important modification of the ground-plan is the formation of a massive xylem-bridge between the ingoing and outgoing branches of  $P$  and  $P'$  (Diagram III, Text-fig. 16, and Pl. IX, Fig. 3). This bridge is very conspicuous in all the seedlings, and it must be slightly arched, for in serial sections followed downwards xylem elements commonly appear in the gap opposite  $M$  at a level higher than that in which the coleoptile traces begin to divide. As no formation of this kind is found in *Zizania*, where the division of the coleoptile traces between the stele and the scutellum trace is particularly clear, we are inclined to think that the formation of a xylem arch is not a primitive character. We have already (p. 167) suggested a possible function for it in *Avena*—to supply the scutellum with water from the lower roots by a shorter path than would otherwise be available. In the aquatic *Zizania* this adaptation is unnecessary.

The stele of the mesocotyl is now complete (Pl. IX, Fig. 5). It is built up of the midrib trace  $M$  from the first leaf, of two plates ( $rp.$ ,  $rp.$ ), on which the nodal roots have been inserted, and of trace  $x$ , derived from the ingoing branches of the coleoptile traces. The root-plates when clear of the nodal roots lose their external xylem, but are always distinguished by a chain of large vessels.

As soon as the nodal roots ( $r.$ ,  $r.$  in Pl. IX, Fig. 4) have differentiated their steles, they are in direct connexion through the root-plates with the main traces of the second and third leaves—already fully formed at that epoch. The first leaf is connected with the root-plates through a pair of its smaller lateral traces only ( $L_1$  and  $L'_1$ ), and perhaps by a few stray xylem elements from other traces as mentioned above. The main traces of the first leaf, together with a portion of each coleoptile trace, pass down the stele of the mesocotyl into the primary root. We have already shown how the xylem arch puts the scutellum also into direct communication with the primary root and the lower cauline roots. Thus the scutellum, coleoptile, and first leaf are dependent throughout their lives on the lower root-system, consisting of the primary root and three insertion roots (Text-fig. 12, p. 171). The second and third leaves are also connected with this lower root-system through the root-plates, for the latter bear the insertion roots below as well as the nodal roots above. Indeed, since the nodal roots do not become functional until the second and third leaves are unfolded, these leaves too draw water in their youth from the insertion roots.

Little remains to be said concerning the lower root-system. Its relation to the mesocotyl has already been described. The primary root is heptarch or octarch; the insertion roots hexarch or heptarch.

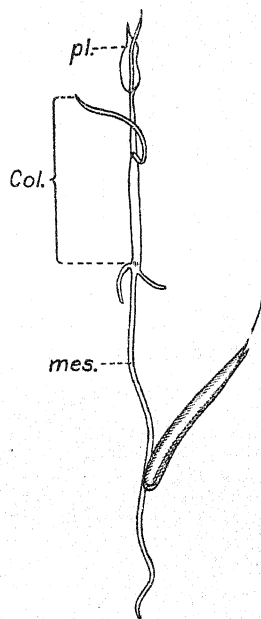
*Avena* itself is, as we have already said, the most complete specimen of its type. *Zizania aquatica*, L., the Manchurian Water-Rice, deserves special attention on account of the simple structure of its first node, in which we think it approaches the primitive type. We have also described two other Grasses of the *Avena* type for comparison: *Lolium italicum*, A. Br., and *Leersia oryzoides*, Sw.

*Zizania aquatica*, L. The fruits are long and slender. They will germinate only while quite fresh, and when planted under water. The vascular ground-plan of the seedling is like that of *Avena*. The numerous differences in detail can generally be referred to the adaptation of *Zizania* to an aquatic habit.

The endosperm of *Zizania* is scanty compared with that of *Avena sativa*, in which—as in all cultivated cereals—it is unnaturally bulky. The scutellum is like a linear leaf in outline. It is separated from the coleoptile by a very long and slender mesocotyl (Text-fig. 18, and Pl. IX, Fig. 6). As in *Avena*, the scutellum trace runs upwards to the first node side by side with the mesocotylar stele, but is perfectly distinct from it throughout.

The stem-bud is wrapped in a linear leaf-like coleoptile; its margins united into a short tubular sheath at the base (Pl. IX, Fig. 6). There is no stiff pointed cap such as that which protects the early leaves of *Avena* and other terrestrial Grasses. But two bundles run nearly the whole length of the coleoptile, and, seen in transverse section, they occupy the same relative positions as in *Avena*, and recall the two-nerved 'axillary stipules' found in some species of *Potamogeton*.<sup>1</sup>

We consider the mesocotyl of *Zizania*, like that of *Avena*, to represent a fusion of the cotyledonary stalk with the hypocotyl. If we mentally reconstruct a form in which they were separate

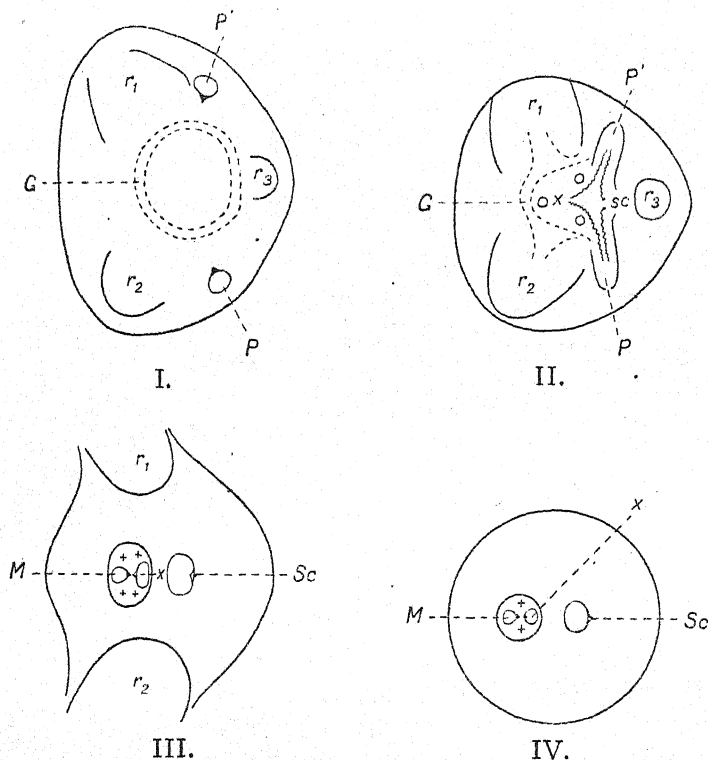


TEXT-FIG. 18. *Zizania aquatica*, L. Seedling, life-size.

<sup>1</sup> Cf. the description given by E. Cosson (Note sur la stipule et la préfeuille dans le genre *Potamogeton*, et quelques considérations sur ces organes dans les autres monocotylées. Bull. de la Soc. Bot. de France, t. vii, p. 715, 1860): 'La stipule, dans la plupart des *Potamogeton*, est constituée par un organe indivis, membraneux, libre, de forme et de longueur variables, inséré à l'aisselle de la feuille, à face supérieure regardant du même côté que la face supérieure de la feuille correspondante, et entourant d'une manière plus ou moins complète la base de l'entre-nœud de la tige. . . Cette stipule axillaire présente, surtout dans les espèces à feuilles pétiolées, deux nervures presque parallèles, saillantes, généralement en forme de carène, placées exactement à la limite du point de contact du pétiole. Assez souvent cette stipule n'est binerviée et bicarénée que dans sa partie inférieure, et quelquefois même elle n'est nullement binerviée.'

members, we find them meeting each other and the coleoptile at the first node. Then the coleoptile of such a form would clearly bear the same relationship to its cotyledon as the 'axillary stipule' of *Potamogeton* to its own leaf.

The epiblast is membranous and very well developed (Pl. IX, Fig. 6). The primary root is less vigorous than that of *Avena*, and no cauline roots



TEXT-FIG. 19. *Zizania aquatica*, L. Structure of axis in seedling shown in a series of diagrams representing transverse sections from above downward. I. The coleoptile bundles  $P$  and  $P'$  are shown in the cortex.  $G$  = root-girdle;  $r_1$ ,  $r_2$ ,  $r_3$ , adventitious roots. II. The coleoptile bundles are dividing and fusing to form the scutellum bundle  $sc$ , and the bundle  $x$ , which passes down the mesocotyl in the stele. III and IV. Mesocotylar stele completely formed.  $M$  = midrib of first leaf.

are formed from the insertion zone just above it. They are produced, however, at all the early nodes, which are crowded together at the base of the plumule.

The scutellum contains a single slender bundle, running down the dorsal ridge to the zone of insertion. Here, as in *Avena*, the scutellum bundle enters the axis and at once turns sharply upwards, travelling to the first node side by side with the stele. Throughout its course in scutellum



and axis the bundle is enclosed in an endodermis. The single group of xylem is composed of a few tracheides, often hardly lignified at all. They are commonly annular, but occasionally spiral. In the scutellum the xylem group is turned towards the ventral surface; in the axis it is inverted—that is, directed away from the centre (Text-fig. 19, III and IV). The phloem is massive throughout, but appears single except just below the node, where the trace is prepared to branch to right and left as it enters the stele.

The structure of the first node is obscured by the formation of a vascular girdle (Pl. X, Fig. 9), on which the cauline roots are inserted (*G* in Diagram I, Text-fig. 19). The plumular traces lose their identity in this girdle, but some of them reappear just as the coleoptile traces *P* and *P'* are entering the stele (Diagram II, Text-fig. 19). One trace is opposite the gap which opens to receive *P* and *P'*, and must represent midrib *M* from the first leaf.

Above the cylindrical base of the coleoptile is a region where it shows in transverse section as a crescent on one side of the stem-bud. Here the two bundles are symmetrically placed as regards the outline of the crescent, but not at either end of a diameter of the axis as in *Avena*. They both lie on the same side of this diameter; that side which is opposite to the midrib of the first leaf. At this level the xylem of each bundle consists of a few narrow elements but little lignified. In transverse section the outline of the phloem is almost kidney-shaped, but there is no definite division into two groups. This structure is continued downwards, and—when the coleoptile traces enter the axis—preparations can be found in which the central xylem strand is seen to be bordered on either side by phloem elements (Diagram II, Text-fig. 19).

The coleoptile traces *P* and *P'* approach the stele in opposite directions. They commonly travel along the same straight line, which is approximately tangential to the circumference of the stele at the point where a gap has appeared in the root-girdle (Diagram II, Text-fig. 19). Each has already split into two branches, one running outwards, and the other keeping nearly to the original line of approach (Pl. IX, Figs. 7 and 8). In the three younger seedlings examined, the inner branch of *P* on its way to the gap is seen to unite with the lateral plumular trace adjacent to it, and the inner branch of *P'* carries off the corresponding trace on the other side of the stele. These junctions are masked in the two older seedlings by the insertion of numerous cauline roots. The inner branches unite to form trace *x*, which fills up the gap in the stele. The outward branches form the inverted scutellum trace *sc*. (Diagrams II and III, Text-fig. 19; Pl. IX, Figs. 7 and 8).

The reader will naturally conclude from the above description that the node of *Zizania* resembles that of *Avena*, except that its structure is less clear, and that this lack of clearness is due partly to the reduction of

vascular tissue consequent on the aquatic habit, and partly to the complications introduced by the number of roots found at the first node, and by the formation of a vascular girdle on which they are inserted. On the whole this is true, but in one important character the nodal structure is clearer in *Zizania* than in *Avena*, namely, in the insertion of the scutellum trace. It divides into two branches before reaching the stele. Each branch joins a coleoptile trace. No xylem arch is formed as in *Avena*, and there is therefore no direct connexion between the scutellum trace and the stele. One or two xylem elements may perhaps be found by diligent search to go from one to the other, but the rule is that the scutellum trace divides itself completely between the coleoptile bundles  $P$  and  $P'$ . The other elements of  $P$  and  $P'$  are prolonged downwards into the stele as the compound trace  $x$ .

When the mesocotylar stele is fairly formed, it contains the trace  $x$  derived from coleoptile traces, and a trace opposite to it which probably represents  $M$ , the midrib of the first leaf (Diagrams III and IV, Text-Fig. 19, p. 180). There are also one or two xylem groups on either side which probably correspond to the lateral xylem plates in *Avena*. These lateral groups become insignificant as we descend the mesocotyl, and are very soon reduced to two—rarely three or four—lateral vessels of large lumen, placed symmetrically with regard to  $M$  and  $x$  near the periphery of the stele.

The mesocotylar stele is very little lignified, except just below the first node, and again in the region of transition to a root-structure. Even at this lower level, very accurate staining is required to bring out the xylem elements at all in contrast to surrounding tissues. In two seedlings only among the five examined is it possible to make out the symmetry of the root-steles. In both cases that of the primary root is pentarch, with an inclination to become tetrarch by fusion of two xylem rays. The cauline roots are 6 to 10 arch. The method of transition is obscure in all the seedlings.

Thus the vascular structure of *Zizania* may be described as that of a slender *Avena*, with imperfect lignification and no cauline roots springing from the insertion level of the scutellum. The imperfect lignification is, no doubt, connected with its aquatic habit. Whether the absence of insertion roots is a consequence of this habit too can hardly be determined without observations on the growth of *Zizania* under natural conditions.

The most important anatomical character from our point of view is the very clear way in which the scutellum trace is built up from portions of both coleoptile traces, and the absence of direct connexion between it and the stele of the mesocotyl. If the object of the xylem arch in *Avena* be to put the scutellum into direct communication with the water-supply from the lower root-system (*ante*, p. 167), then the absence of this character is readily

understood. For as the seed of *Zizania* will only germinate under water, the scutellum does not require special adaptations for rapid water conduction.

*Leersia oryzoides*, Sw. The three seedlings which we have examined are all about the same age. The first and second leaves are free from the coleoptile, and the second is unrolling as it leaves the shelter of the first leaf. In this species the first leaf is reduced to a membranous sheath, but the second is a fully-formed foliage leaf.

The species is aquatic, and the seedling resembles that of *Zizania* in general habit. The chief points of difference, excluding the smaller size, are the reduction of the first leaf to a sheathing organ, and the formation of cauline roots from the insertion zone as well as at the first node. It will be seen that the anatomical differences between *Leersia* and *Zizania* are correlated to these external features.

The coleoptile is open and unstiffened; the epiblast leaf-like, and the mesocotyl slender. The primary root is developed much earlier than any cauline root, and is freely branched.

The bundle of the scutellum is slender. It enters the axis at the apparent insertion, and turns sharply upwards without joining the stele, as in all seedlings of this type.

Just above the first node six lignified plumular traces form a single circle in the axis; outside these are the traces from the coleoptile. The three traces from the second leaf are much larger than those from the first, probably because the second is a foliage leaf while the first acts only as a sheath. A xylem girdle is formed here, as in *Zizania*, in which the identity of all the plumular traces is lost. A gap appears in the girdle just before the coleoptile traces have reached the stele. They run in from either side along a tangent touching the stele at this gap.

As the traces approach the stele, the xylem of each divides; one branch entering the stele, the other remaining outside it. The inward branches unite to form a xylem group within the stele, which does not long retain its identity. The outward branches meet outside. The phloem of each coleoptile trace is already double; one group accompanies the inward, the other the outward branch. Thus a double phloem group is formed within and without the stele, but both become single almost immediately. The complete bundle outside the stele is of course the inverted scutellum trace. As in *Zizania* it is not directly connected with the stele by xylem elements.

The stele of the mesocotyl just below the first node is slender but very well differentiated. The cells of the endodermis are thickened, especially on the inner and radial walls. The pericycle consists of a single row of thickened elements, some of them lignified. The later xylem plates of

*Avena* are represented by one or two large pitted vessels on either side of the stele. Between them is a group of small well-lignified xylem elements common to the two phloem groups. This compound structure, two phloem groups opposite to one another and divided by a common xylem group, represents the two traces  $M$  and  $x$  in *Avena*.

Lower down in the mesocotyl the stele loses in differentiation. The endodermis and pericycle are little thickened, and not lignified at all. The central xylem group is only partially lignified. The large lateral vessels are comparatively well differentiated and completely lignified throughout their course, doubtless because—as in *Avena*—they connect the cauline roots of the insertion region with those of the node.

The process of transition to a root-structure cannot be followed in any of our preparations, nor is the symmetry of the primary root to be made out.

*Lolium italicum*, A. Br. In the two seedlings examined, the first leaf has lately emerged from the coleoptile, but one of them is rather further developed than the other. The type is that of *Avena*, but the seedlings are smaller in every dimension; the grain shorter in proportion to its breadth, and the axis more slender. A small epiblast is inserted opposite the scutellum, and at about the same level. Cauline roots are also formed there, and they begin to show externally about the time when the first leaf appears, while at the same epoch the nodal roots are still mere rudiments within the cortex.

The bundle of the scutellum is single. Its xylem is massive, and gives off a few lateral branches near the apex. Below this, the xylem forms a ventral crescent embracing the phloem. At the insertion of the scutellum its trace enters the axis, and at once turns sharply upwards, but it does not join the stele below the first node. A transverse section through any part of the mesocotyl shows the scutellum trace side by side with the main stele, and inverted—that is to say, the xylem of the scutellum trace is directed away from the centre of the stele.

At the first node three plumular traces only can be followed with certainty into the stele of the mesocotyl. They all belong to the first leaf, and represent  $M$ ,  $L_2$ , and  $L'_2$  in *Avena*. Five more are present, though unlignified, above the entrance of the coleoptile traces; they represent  $L_1$  and  $L'_1$  from the first leaf, and  $m$ ,  $l_2$ , and  $l'_2$  from the second. They behave exactly like the corresponding traces in *Avena*.  $m$  divides into two branches which travel to opposite sides of  $M$ ; one uniting with  $l_2$  and  $L_1$  on the way, the other with  $l'_2$  and  $L'_1$ . But all these traces disappear before those from the coleoptile run into the stele. In older seedlings they would doubtless persist, and then their connexion with the lateral xylem plates of the mesocotyl would appear.

Two other plumular strands are perceived in the stele for the first time as the coleoptile traces enter it. They represent  $L_3$  and  $L'_3$ . Trace  $P$  makes its way between  $L_2$  and  $L_3$ , and trace  $P'$  between  $L'_2$  and  $L'_3$ , just as in *Avena*. Even the xylem arch is present, though far less massive.

The large xylem vessels of the lateral plates make their appearance to right and left of  $M$  during the formation of the mesocotylar stele. At first they are numerous and poorly lignified. Lower down, where the stele has assumed its final form, each lateral plate consists of three or four large lignified elements.

The structure of the first node in *Lolium italicum* is very clear because the nodal roots are mere rudiments without vascular tissue at an age when the stele of the mesocotyl is fully defined, and some of the plumular traces are lignified. The lower cauline root-system is also tardy in development, and the transition from mesocotyl to primary root can therefore be followed better in the stele of *Lolium* than in that of *Avena* or *Zizania*. The details, however, are not very clear. In one seedling—the younger of the two—the xylem breaks up into four crescents, convex towards the centre. Protoxylem elements are found at their extremities, and the result is a pentarch root-stele. In the older seedling there are at first three xylem groups, not definitely crescent-shaped. Here, too, the stele of the primary root becomes pentarch.

#### B. *Zea* type.

*Sorghum vulgare*, Pers. The seedling structure of this species is clearer than that of *Zea*, because the formation of cauline roots is delayed until the appearance of the second leaf, both at the first or plumular node; and at the insertion level. We have therefore chosen this species for detailed description, and it will represent the type named from the better-known genus *Zea*.

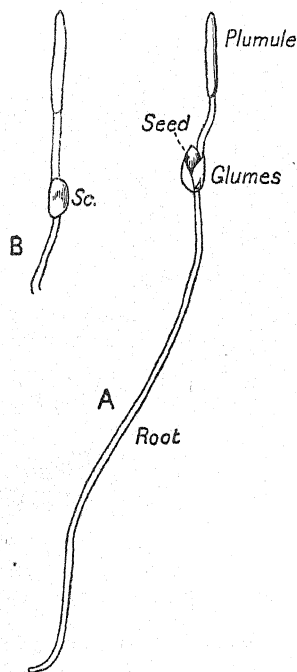
The seedling drawn in Text-fig. 20 A, p. 186, has just reached the age when the first leaf is about to push through the slit near the tip of the coleoptile. This still sheathes the stem-bud, and is borne on a fairly long mesocotyl. The primary root is long and unbranched. There are as yet no cauline roots.

We have examined the vascular skeleton of five seedlings, including that mentioned above. Two of the others are younger, and two older. The oldest shows two foliage leaves, but no cauline roots are visible externally. The vascular structure in all five seedlings is singularly uniform.

The scutellum has a single bundle with massive phloem, but comparatively little lignified xylem. In the upper part, branches are given off freely towards the dorsal surface of the scutellum, which is covered by an active epithelium. In this region the xylem elements are scattered about the periphery of the main bundle, but even here they are far more

frequent on the ventral side, and lower down—where branches are fewer—they form a ventral crescent. Still lower, no branches are given off from the main bundle, and the xylem elements are collected into a compact ventral group.

The scutellum trace does not run up the mesocotyl side by side with the stele as in *Avena* or *Zizania*, but enters it at once. Its elements, however, can be followed within the stele above its insertion. Below that level



TEXT-FIG. 20. *Sorghum vulgare*, Pers. A. Seedling, life-size. B. Upper part of same seedling with grain removed to show scutellum.

there is a complex formation of xylem and phloem resembling that found in *Avena* in the region from which the lower cauline roots are given off. The identity of the mesocotylar traces is lost in this plexus, which we have called the insertion plate. In the oldest seedling which we have cut, no cauline roots are formed at this level, but they may be present in seedlings older still. Below the insertion plate, the axis is abruptly transformed into the primary root. Above it, the mesocotylar stele soon acquires its characteristic structure, and preserves it almost unchanged up to the plumular node. For convenience we must first describe the complete stele above the insertion, and return later to the entrance of the scutellum trace, though this reverses the natural sequence of events.

The whole stele is surrounded by a very well-marked endodermis. The internal wall of each cell in this layer is thickened and lignified; but the radial walls are little thickened, though more or less completely lignified (Pl. X, Fig. 10). Within the endodermis are at least two layers of thick-walled but unlignified cells.

The vascular tissue of the stele consists of three phloem centres, each defined internally by a xylem crescent, and externally by the thick-walled peripheral tissues. One of these phloem centres (*M*) differs from the other two (*P*, *P'*) in containing a single group of soft bast. The lignified xylem crescent embracing this phloem group is broken, but clearly indicated. Its protoxylem elements ( $\phi x_3$ ) are distinct, and nearer the centre of the stele than the rest of the crescent. At either extremity are one or two large well-lignified elements, but no trace of protoxylem. The whole structure forms a typical endarch bundle, which we call the median trace to distinguish it from the two lateral traces *P* and *P'*.

Each of the lateral traces is separated from the median trace by an unthickened element of very large lumen ( $rx.$ ,  $rx.$ ) resembling the large vessels characteristic of monocotyledonous roots. No such element occurs between the lateral traces. They are separated by several layers of ordinary conjunctive tissue. In transverse section this appears as a broad pathway of clear tissue opposite the protoxylem of the median bundle ( $px_3.$ ). Within this pathway, near the periphery, is a single lignified element of protoxylem ( $px_1.$ ), to which we shall refer later (Pl. X, Fig. 10).

The lateral bundles are alike, and each differs from the median bundle chiefly in the fact that its metaxylem crescent embraces two groups of soft bast instead of one. These groups are partially separated from each other by a projection of the peripheral thickened layers. A little group of protoxylem elements ( $px_2.$ ), sometimes reduced to a single lignified tracheide, is common to both crescents, and at the extremities of both there are large thick-walled vessels. Such vessels are also found at the ends of the median crescent *M* (Pl. X, Fig. 10).

The characteristic structure just described is found in the mesocotylar stele of the five seedlings examined. In all of them the scutellum trace is found to be connected with the lateral bundles only. Its scanty xylem is represented in the stele by the isolated element of protoxylem ( $px_1.$ ) already described as occurring between the two lateral crescents. It is not always easy to identify this solitary element. When the thickening is annular, it can be picked out only in sections which include a ring or part of one, and the imperfectly lignified rings do not stain deeply. In three of the seedlings examined the whole mesocotyl, from insertion to first node, is included in a single series; and we have satisfied ourselves that the chain of single protoxylem elements can be followed from one end of the series to the other. In the two remaining seedlings, the mesocotyl was divided before microtoming, and accordingly we have two separate series in each: one passing through the insertion of the scutellum, and a second—with different orientation—through the first or plumular node. The scutellum xylem within the mesocotylar stele is represented in both insertion series by a single peripheral tracheide, which is either spiral or annular, and is always isolated in the conjunctive tissue separating the lateral bundles. In both nodal series a similar element is found in the corresponding position, and successive elements can be followed upwards to the plumular node.

The phloem of the scutellum trace is massive. It divides, travelling to right and left of its scanty xylem on entering the stele. Below this level all traces are confounded in the insertion plate, but it is pretty clear that the scutellum trace contributes only a sharp elbow of tissue to this structure. Its real course lies up the stele, as already described for its xylem. The phloem branch on either side is absorbed in a lateral phloem centre of the mesocotyl. During the junction there is no clear division between the two

groups of soft bast which constitute a centre. The outline of the double group is kidney-shaped, but the phloem elements of the scutellum trace seem to run chiefly into the nearer of the two segments. When these have finally separated, the xylem crescents are also defined, and the mesocotylar stele has assumed its characteristic structure (Pl. X, Fig. 10).

Thus the scutellum trace supplies a single isolated element of protoxylem ( $px_1$ ) to the mesocotylar stele, and also contributes at least half of the phloem to each of the two lateral centres. It does not account for all the lateral phloem, nor for any part of the lateral xylem crescents, and it is quite unconnected with the median bundle.

Below the scutellum insertion the vascular symmetry of the mesocotyl is sharply separated from that of the primary root by the formation of a plate of vascular tissue. In the upper part of this region the phloem of the median bundle can still be traced distinctly. Opposite to it is a single phloem group, representing the elbow of the scutellum trace as it turns on itself. The massive xylem crescents which define these two groups internally embrace and nearly meet round them. The centre of the stele is occupied by a plate of metaxylem uniting the crescents with each other. This formation recalls a similar structure in the transitional region of *Fritillaria imperialis*,<sup>1</sup> L. It persists through a few sections only. Below these the phloem and xylem become inextricably mingled, and finally settle down into normal root-structure.

The primary root is polyarch, with nine or ten xylem rays. Just within the protoxylem of each ray is a single well-lignified element of wide lumen. When cut obliquely it is seen to be a vessel with pitted walls. The ray commonly ends with this vessel, but sometimes a few smaller lignified elements lie next it on the inner side.

In the centre of the root-stele are many xylem elements scattered among unthickened cells which probably represent conjunctive tissue. These xylem elements are of different sizes; their walls more or less thickened, and more or less lignified. They do not form a continuous xylem plate, and are distinct from the rays. Among them are always two elements of very large lumen. Their walls are only slightly thickened; but in the older seedlings examined they are completely lignified, and can be seen—where cut obliquely—to be uniformly pitted. Each is bordered by a single row of small elements, mostly unlignified, and some of them thin-walled. The two large vessels can be followed upwards through the insertion node, and are found to be continuous with those which separate the median bundle of the mesocotyl from the lateral bundles ( $rx$  in Pl. X, Fig. 10).

Returning to the stele of the mesocotyl, its structure is slightly

<sup>1</sup> Sargent, E. ('03): A Theory of the Origin of Monocotyledons, founded on the Structure of their Seedlings. *Ann. of Bot.*, vol. xvii, p. 24, and Pl. III, Fig. 4, 1903.



modified as it approaches the first or plumular node. All its elements become less differentiated. The cells of the endodermis and conjunctive tissue just below the node are little if at all thickened; the xylem is less lignified. In particular all the vessels of large lumen become quite thin-walled, and are apt to appear crushed in transverse section. Two phloem groups are found in the median trace, so that but for the two sentinel vessels (*rx.*) there would be some difficulty in distinguishing the median from the lateral bundles.

At the node a number of plumular traces combine with the two traces from the coleoptile to form the stele of the mesocotyl. In none of these traces are any elements of wide lumen present, such as those which extend from the root upwards through the mesocotylar stele. All such elements appear suddenly just below the node.

The coleoptile bundles are distinctly double (Pl. X, Fig. 11). Each consists of two phloem groups embraced by a common xylem crescent. In places two groups of protoxylem are indicated, corresponding to the two phloem groups. At the node the coleoptile bundles run in from opposite sides of the axis in a straight line, which is perpendicular to that bisecting the midrib of the first leaf. The xylem groups of these bundles meet in the centre of the axis, forming a bridge across the node (Pl. X, Fig. 12). The phloem groups of each bundle separate, and one runs in on either side of the xylem.

In the youngest seedling cut, numerous plumular traces are differentiated just above the node. Of these, ten possess at least one lignified xylem element. Five lignified traces are on one side of the xylem bridge, and five on the other. The stele is not quite symmetrically divided however, as on one side the traces are larger than on the other, and more unlignified strands are present. The median trace in the larger segment represents the midrib of the first leaf; it is commonly the largest trace, and always the best lignified. Though at one point its xylem just touches the xylem bridge, it forms a centre independent of the coleoptile traces. Several unlignified strands are inserted on it at the node, and together they form the median bundle of the mesocotylar stele (*M* in Pl. X, Fig. 10). Of the bundles on the same side as the midrib trace, several of the strands, and all four lignified traces, are inserted on the coleoptile bundles; and so are all the traces—lignified and unlignified—on the other side.

In the older seedlings the nodal structure is essentially similar, but the discrepancy in number and size of the traces on either side of the xylem bridge is greater.

The coleoptile traces form the lateral bundles of the mesocotylar stele. The two phloem groups of either lateral bundle are derived from those of a single coleoptile trace. The xylem bridge breaks up into two groups, each of which—supplemented by the large vessels derived from the

root—forms a lateral xylem crescent. The single element  $px_1$ , which becomes visible in the peripheral gap between the lateral traces, is derived from the xylem bridge too.

The vascular elements of the mesocotylar stele in *Sorghum* are thus derived from three distinct sources :

1. The phloem of the median bundle ( $M$  in Pl. X, Fig. 10), and most of its xylem, is plumular; for it is derived from the midrib of the first leaf and the plumular traces inserted on it above the node.

2. The phloem of the two lateral bundles, and much of their xylem, is derived from the coleoptile bundles, and the numerous plumular traces inserted on them. The symmetry of each lateral bundle reproduces that of a coleoptile bundle; there are two phloem groups and a common xylem crescent in one as in the other. The single element  $px_1$ . (Pl. X, Fig. 10) also comes from this source.

3. A certain number of large xylem elements, forming part of the lateral crescents, are derived from the root-xylem and disappear at the node. In particular the two 'sentinel' vessels ( $rx$ ,  $rx$ , in Pl. X, Fig. 10), which divide the median from the lateral bundles, can be traced throughout the mesocotyl into the root.

#### *Comparison of Sorghum seedling with that of Avena.*

There is of course no doubt as to the complete homology of scutellum, coleoptile, and primary root in the two seedlings. The real question is whether the mesocotyl of *Sorghum* is homologous with that of *Avena*. If it is not, what member or members of a typical monocotyledonous seedling does it represent? We have already explained that we consider the mesocotyl of *Avena* to represent a fusion of the cotyledonary stalk with the hypocotyl (*ante*, p. 165). If, on the other hand, the mesocotyl is morphologically equivalent in both seedlings, how account for the differences in vascular anatomy?

Adopting the latter view, we believe that the mesocotyl of *Sorghum* is equivalent to the mesocotyl of *Avena*, and we have therefore to explain its anatomy on that assumption. The most obvious difference is that in *Avena* the scutellum trace joins the stele of the axis at the top of the mesocotyl; that is, at some distance above the apparent insertion of the scutellum. Between these two levels the scutellum trace runs upwards in the cortex of the mesocotyl; side by side with the stele, but quite distinct from it (Text-fig. 6, p. 166). But in *Sorghum* the scutellum trace goes straight to the stele as soon as it enters the axis; the real insertion appears to lie in the same plane with the apparent. Our explanation is that the fusion of stalk and hypocotyl has proceeded further in *Sorghum* than in *Avena*; the cotyledonary trace in the former runs up to the plumular node *within* the stele, not outside it.

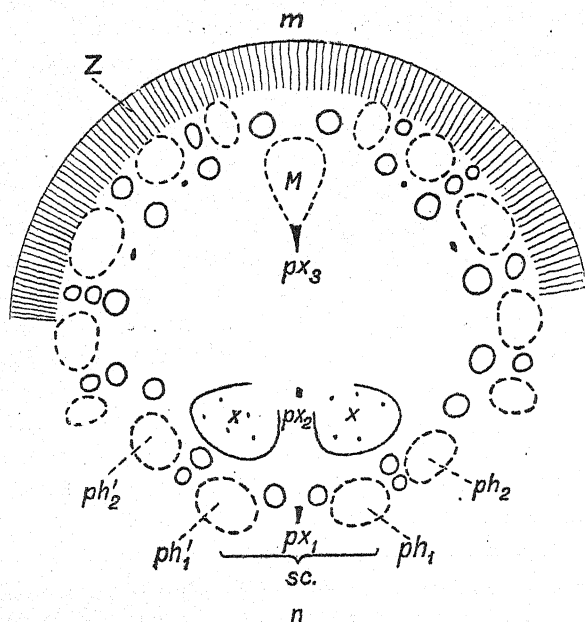
We have attempted to explain the vascular symmetry of the *Avena* seedling by supposing that the scutellum trace divides at the plumular node; that one branch runs up one side of the coleoptile, doubles closely on itself near the top, and runs down again, thus forming one of the double coleoptile bundles. The other branch does the same on the other side. When they reach the plumular node again, each downward branch plunges into the stele of the mesocotyl. Here they unite to form a compound bundle opposite the midrib trace *M* (Pl. IX, Fig. 4, also *ante*, pp. 167-9, and Text-figs. 6 and 7, p. 166). To explain the vascular skeleton of *Sorghum* in the same terms, we must consider the solitary element  $px_1$  as representing the xylem of the upward scutellum trace in *Avena*, and the two phloem groups adjacent to it—within the lateral xylem crescents—as its phloem. At the plumular node all these structures are seen to be connected with half of each coleoptile trace, just as in *Avena*; while the remaining phloem group within each lateral crescent belongs to the other half of the corresponding coleoptile trace, and can be traced downwards through the plumular node into the stele of the mesocotyl.

Those who adopt this view will find that the chief difficulty to be faced is the existence of an insertion plate at the point of junction of the scutellum trace with the stele. For if the scutellum is identified with the cotyledon, the level where its trace is inserted on the stele would appear at first sight to be undoubtedly the cotyledonary node. But then the mesocotyl of *Sorghum* would represent the first internode of the main axis, and this is contrary to our hypothesis. Nor is it consistent with our observations, for we have shown that the structure of the upper or plumular node of *Sorghum* is strictly comparable with that of the first or plumular node in *Avena*, where the scutellum trace first unites with the stele. On the other hand, the insertion 'node' in *Sorghum* resembles with equal fidelity the region in *Avena* where the lower cauline roots are given off. We therefore prefer to use the term 'insertion plate', and we continue to regard the node where the coleoptile traces enter the stele as the first node of the axis in *Sorghum* as in *Avena*. It is strictly comparable in our opinion with the node which in a typical Monocotyledon divides the hypocotyl from the first internode of the plumule.

*Zea Mays*, L. We have already described and figured the ramifications of the single bundle in the upper part of the scutellum (Sargant and Robertson ('05)). We may therefore begin here with the structure of that bundle near the base, just before it enters the axis. At this level it is oval in transverse section, with massive phloem (Text-fig. 21, p. 192). Its xylem is compact and well-lignified. The xylem divides into three parts while passing into the axis, and the phloem into two. The lateral branches of the xylem enter the two cauline roots which are inserted between scutellum and

axis—the 'wedging' roots as we have called them (l. c., Pl. V, Figs. 6-9, *r'*). The median segment is the most slender of the three. It goes straight to the axial stele, and at once turns upwards within it. The two phloem strands accompany the scutellum xylem, one on either side of it. Each gives off a considerable portion to the stele of the nearer wedging root on its way to the axial stele. Thus the scutellum xylem enters the latter with a phloem branch on either hand, and they turn upwards side by side with it.

These three strands, one of xylem and two of phloem, can be followed from their entrance into the mesocotyl upwards to the first node in



TEXT-FIG. 21. *Zea Mays*, L. Diagram of mesocotyl as seen in transverse section.

two seedlings. Both of them are young, with the stem-bud still completely enclosed in the coleoptile. The cauline roots which will be produced later from this node are not present even as rudiments, though a band of meristem partly enclosing the stele suggests the formation of new members there. So far, indeed, the youth of the seedling is an advantage, since it allows the primary structure of the node to be followed without the complications introduced by root-insertions. But on the other hand the mesocotylar stele is very imperfectly differentiated at this age. After very careful inspection of these two complete series, and comparison with several partial ones from seedlings of the same age or older, we found certain landmarks within the stele which enabled us to interpret its structure

with a fair degree of probability. Only after comparison with the allied case of *Sorghum*, however, which in some respects is much more favourable for observation, were we fully convinced that this interpretation was sound.

The first fixed point is the little group of protoxylem elements, sometimes even reduced to a single annular tracheide with a phloem group on either hand, which represents the xylem of the scutellum trace. In the diagram (Text-fig. 21) it is marked  $px_1$ , and the double bundle consisting of  $px_1$  and the adjacent phloem strands is called  $sc.$ , because it answers to the upward scutellum trace in *Avena*. In series which include the scutellum insertion, this group ( $px_1$ ) can of course be determined with absolute certainty. But even in an isolated section of the mesocotyl, the group  $px_1$  can be picked out, partly by its position near the periphery of the stele, and with still more certainty by reference to a second fixed point, the midrib trace from the first leaf ( $M$ ). In *Zea Mays*, as in the other Grass seedlings examined, the midrib of the first leaf is prolonged almost unchanged into a trace which can be followed with certainty throughout the mesocotyl. It lies a little nearer the centre of the stele than the other traces, and its xylem is better lignified. The group  $px_1$  is always at the further end of the diameter bisecting  $M$ .

The rest of the stele is symmetrical with respect to this diameter. The phloem group on either side of  $px_1$  is followed by another phloem group resembling it. Internal to the pair of phloem groups thus lying on one side of  $px_1$  is a wedge-shaped area, over which are scattered xylem elements more or less completely lignified. A similar area is found on the other side. The two wedges ( $x$ ,  $x$  in Text-fig. 21) are separated by a lane of clear conjunctive tissue, closed at the peripheral end by  $px_1$ , and between the apices of the wedges by another little group of protoxylem elements ( $px_2$ ). Thus the diameter  $m n$ , which bisects  $M$ , also passes through three distinct protoxylem groups:  $px_3$  belonging to  $M$ ;  $px_2$  common to the two areas  $x$ ,  $x$ ; and  $px_1$  belonging to the scutellum trace.

The numerous lateral plumular traces lie near the periphery of the stele. They occupy the two arcs which are bounded by  $M$  on one side, and on the other by either extremity of the cotyledonary bundles which face it. They are best defined in young seedlings by their phloem groups, for only a xylem element here and there is sufficiently well lignified to show up clearly in transverse section. Nevertheless, the existence of such elements within the larger phloem groups shows conclusively that the lateral traces from the first leaf are endarch, like their medium trace  $M$ .

Between the phloem groups of plumular and cotyledonary traces alike are large elements, lignified in older seedlings, which clearly correspond to the root xylem found in the mesocotyl of *Sorghum*. They extend in places to the periphery of the stele. Since these are by far the most conspicuous xylem elements present in the section, and since among them the smaller

elements are commonly external to the rest, it is not surprising that the mesocotylar stele of *Zea* has been described as root-like.<sup>1</sup> This is, however, a mistake. The plumular traces, as well as those connected with scutellum and coleoptile, are certainly of the usual stem-type; that is, their xylem is centrifugal, and internal to the phloem.

We have now described the anatomy of the mesocotylar stele near its base, and its connexion with the scutellum trace. Following it upwards, we find some changes in a transverse section taken just below the first node. Much of the root xylem has died out, and what is left is thin-walled and unligified. The two phloem bundles on either side of  $px_1$  ( $ph_1$ ,  $ph_2$ ,  $ph'_1$ , and  $ph'_2$ ) sometimes divide into three or four groups. In one seedling two small groups, one on either side of  $px_1$ , appear to represent a pair of minor plumular bundles whose insertion on  $ph_1$  and  $ph'_1$  respectively has been delayed for some distance below the node. The two or three groups beyond them represent  $ph_1$  and  $ph_2$  on one side, and  $ph'_1$  and  $ph'_2$  on the other.

Here, as in *Sorghum*, the first node is most easily described from above downwards. The plumular traces are very numerous, even in these young seedlings, but mostly small and unligified. The midrib  $M$  of the first leaf can be traced with certainty through the complications of the node into the mesocotylar stele. The other traces anastomose with each other, and most of them finally settle down into the single semicircle shown in Text-fig. 21. A few insert themselves on the coleoptile traces at or near the node.

The coleoptile traces themselves are clearly double, with two well-marked phloem groups side by side, and groups of xylem internal to both. There is a common group of protoxylem, and lignification of the metaxylem seems to start on either side of it, suggesting that in older seedlings the metaxylem will be in two groups like the phloem. The traces do not divide until they have entered the stele. They approach each other from opposite directions in the same straight line; so that the two larger xylem branches, maintaining their original direction, meet to form a massive xylem bridge, which divides the circular stele into two unequal segments. The two smaller xylem branches bend outwards, and meet almost at once to form the peripheral protoxylem group  $px_1$ .

We have already described the mesocotyl just below the node in one seedling. In this instance the two phloem groups of each trace, together with the plumular phloem inserted on them, give rise to three or four groups in the stele just below the node. Lower down these unite to form the two groups  $ph_1$ ,  $ph_2$ , on one side of  $px_1$ , and  $ph'_1$ ,  $ph'_2$ , on the other. We do not doubt that the two groups in each pair represent the two phloem groups of a single coleoptile trace, but they do not seem to maintain their

<sup>1</sup> Schellenberg and Kirchner in Kirchner, O. von, Loew, E., and Schröter, C. ('08-'12), p. 231.

independence completely throughout the nodal region. In the two other seedlings from which we possess complete series of sections through the first node, the phloem groups of each coleoptile trace seem prolonged into two groups in the mesocotyl, and to remain fairly distinct during the complications of the first node.

Thus in *Zea*, as in *Sorghum*, the scutellum trace can be followed from its entry into the stele upwards to the first node, where it divides. Each branch forms half one of the coleoptile traces. The other half is prolonged downwards into the stele of the mesocotyl, and is found throughout its course side by side with the upward scutellum trace. Thus from first node to scutellum insertion, a transverse section shows the double scutellum trace, with a trace derived from the coleoptile on either side. Below the scutellum insertion the main axis becomes the primary root. The transition from the stem structure of the mesocotyl to the characteristic structure of the primary root is masked by a very complicated insertion plate. When the stele emerges from this region it is completely root-like.

*Coix Lacryma-Jobi*, L. Of the four seedlings cut, only one is so young that the first leaf is still enclosed in the coleoptile (Text-fig. 22, p. 196). In the others, two leaves have unfolded. The first ( $L_1$ ) is only sheathing; the second ( $L_2$ ) is a foliage leaf (Text-fig. 23, p. 196).

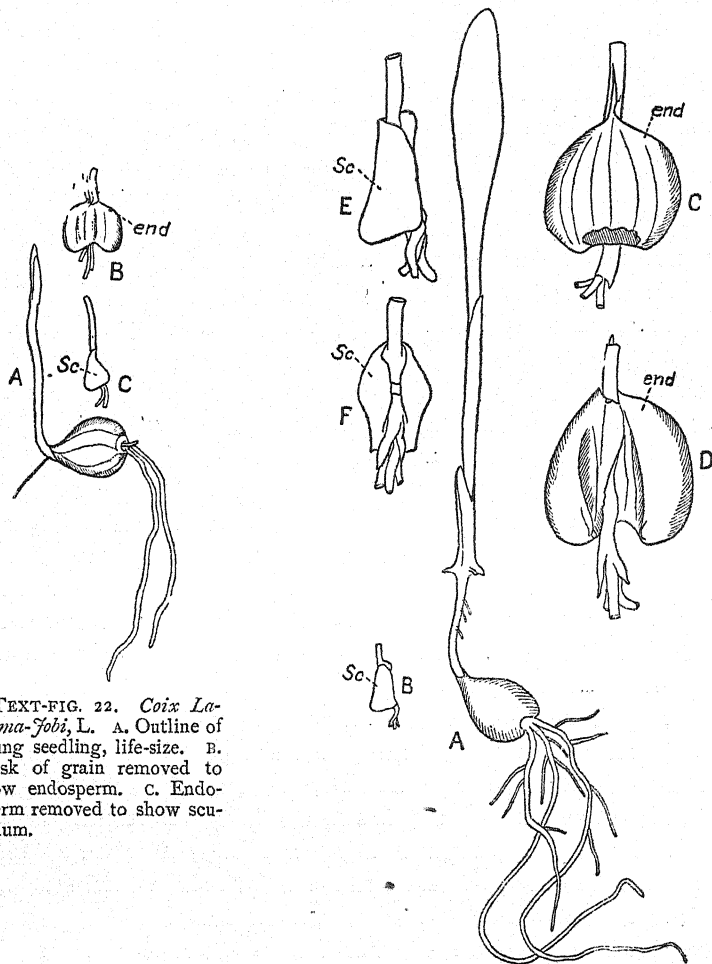
The scutellum is fleshy and wrapped round the axis. Its apparent insertion is prolonged for some distance, but the single massive bundle enters the axis almost at once, and often joins the mesocotylar stele above the level at which its structure becomes obscured by the entrance of steles from the insertion roots. In such cases the xylem and phloem of the scutellum trace can be followed up the main stele with some accuracy.

From apex to insertion the scutellum bundle branches freely, and even as it enters the axis branches are still given off to supply the lower part of the organ. The main trunk becomes amphivasal from constant insertion of branch bundles, and is still amphivasal at its junction with the axis. The external xylem on its dorsal and lateral faces enters the downward branches for the most part, and the main bundle carries nothing but its compact mass of ventral xylem into the mesocotyl.

The phloem of the scutellum bundle is sometimes clearly double and always massive. Its outline in transverse section is kidney-shaped, at any rate near the insertion; the concavity occupied by ventral xylem. On entering the axis it approaches the stele very gradually, so that a section just above their junction may cut both scutellum trace and stele almost transversely.

In such a section the ventral xylem group of the scutellum trace is easily recognized on its upward course in the stele ( $px_1$  in Text-fig. 24, p. 197). The phloem groups on either hand ( $ph_1$  and  $ph'_1$ ) represent those of

the trace. The identity of  $px_1$  with the scutellum xylem can be demonstrated by following sections downwards. That of phloem groups  $ph_1$  and  $ph'_1$  with the scutellum phloem is not quite so clear, because during the trace-insertion, and for some distance above it, the limit between  $ph_1$  and  $ph_2$  on one side, and that which divides  $ph'_1$  from  $ph'_2$  on the other, is confused.



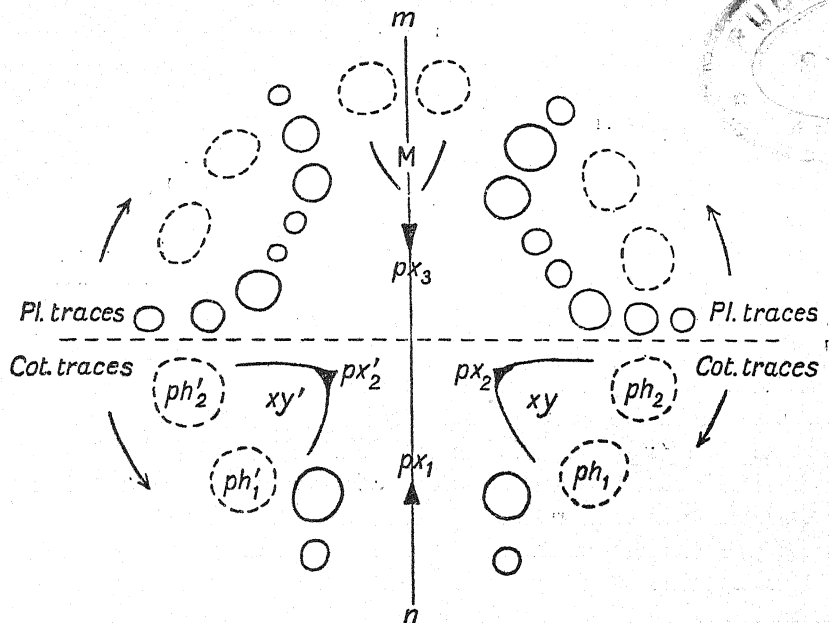
TEXT-FIG. 22. *Coix Lacryma-Jobi*, L. A. Outline of young seedling, life-size. B. Husk of grain removed to show endosperm. C. Endosperm removed to show scutellum.

TEXT-FIG. 23. *Coix Lacryma-Jobi*, L. A. Outline of older seedling, life-size. B. Scutellum and adjacent parts, life-size. C. Endosperm from back.  $\times 2$ . D. Endosperm from front.  $\times 2$ . E. Scutellum from side.  $\times 2$ . F. Scutellum from front.  $\times 2$ .

Starting from  $px_1$  as a fixed point, the structure of the mesocotylar stele is easily deciphered by reference to that of *Sorghum* and *Zea*. It is adequately shown in all four seedlings, and is symmetrical about the



diameter which passes through  $px_1$ . This diameter cuts the similar group  $px_3$ , which lies on the other side of the centre, but nearer to it than  $px_1$ . As in *Sorghum* and *Zea*,  $px_3$  represents the protoxylem of midrib trace  $M$ , and is similarly situated. But *Coix* differs from *Sorghum* and *Zea* in the absence of an intermediate group of protoxylem on this diameter ( $px_2$  in Text-fig. 21). We have interpreted this group in *Sorghum* and *Zea* as common to the two groups of metaxylem on either side of  $px_1$ . In *Coix* each of these metaxylem groups has its own group of protoxylem, placed just within it, at a considerable distance on either side of the diameter of symmetry. The two groups are marked  $px_2$  and  $px'_2$  in Text-fig. 24.



TEXT-FIG. 24 *Coix Lacryma-Jobi*, L. Diagram of mesocotyl as seen in transverse section.

The contribution of the scutellum trace to the stele of the mesocotyl is therefore the protoxylem group  $px_1$ , and part of the two phloem crescents on either side of it. When each crescent settles down into two distinct groups,  $ph_1$  and  $ph_2$  on one side,  $ph'_1$  and  $ph'_2$  on the other, it is natural to identify  $ph_1$  and  $ph'_2$  with the two halves of the scutellum phloem. The other constituents of the stele cannot be identified until they have been followed to the first node.

We have series through the first node from two seedlings only, but both of them are complete and well differentiated. Some obscurity is introduced by the insertion of three cauline roots, but the midrib trace  $M$  can be followed into the stele of the mesocotyl, and the general course

of the coleoptile traces laid down. The latter behave, on the whole, very much as in *Sorghum*. They appear to give rise to  $px_1, px_2, px'_2$ ; to the two xylem groups  $xy, xy'$ ; and to the phloem groups  $ph_1, ph_2, ph'_1, ph'_2$ . But minor plumular traces are inserted on them at critical moments, and further complications are introduced by the formation of cauline roots in the neighbourhood; hence the whole process is obscure, and would hardly be explicable but for comparison with *Sorghum*.

No massive xylem bridge is formed by the union of coleoptile traces. A few elements of protoxylem are pushed forward from either side, and meet in the centre. There they are joined by similar elements from the midrib trace  $M$ . The mass of xylem elements in both traces stop far short of the centre, and form the two groups  $xy, xy'$  with their internal protoxylem. The central group remains undivided for a very short distance, then it splits up into  $px_1$  and  $px_3$  (Text-fig. 24, p. 197).

Except for the temporary union of its protoxylem with  $px_1$ , the midrib trace  $M$  preserves its independence. A number of minor plumular traces are inserted on it at the nodes. In *Sorghum*, as described on p. 190, this trace is the only purely plumular element in the mesocotyl. In *Zea*, on the contrary, there are numerous plumular traces on either side of it. *Coix* is intermediate in this respect. One or two plumular traces appear to be prolonged into the mesocotyl on either side of  $M$ . About half the stele is derived from plumular traces; the other half from cotyledonary (Text-fig. 24, p. 197). The ground-plan of the mesocotylar stele is essentially the same in all three genera, but *Coix* exhibits one feature—the separation of  $px_2$  from  $px'_2$ —which, in our opinion, is primitive.

Cauline roots are given off from the insertion zone as well as at the first node, and also at intervals along the mesocotyl. We were not surprised to find the elements characteristic of root xylem very well developed throughout this region. It is arranged with some regularity, as shown in Text-fig. 24, forming bays and crescents internal to the phloem.

*Euchlaena mexicana*, Schrad. We possess but one series of sections from this species. It passes through scutellum and base of mesocotyl downwards to insertion zone and primary root. The mesocotylar stele resembles that of *Coix*; but the insertion of the scutellum trace on it is much clearer, chiefly owing to the absence of cauline roots in this region. The two phloem groups in the scutellum bundle are quite distinct, and they enter the stele of the mesocotyl on either side of the ventral xylem. As these phloem strands pass up the stele they remain distinct from adjacent strands, and can therefore be identified with certainty as belonging to the scutellum trace.

*Comparison of the Zea type with the Avena type.*

In these two types the scutellum is inserted at some distance below the first node. The region of the main axis which separates them has been called the mesocotyl.

In the *Avena* type the scutellum trace on entering the axis turns sharply upwards in the cortex and does not join the stele until it has reached the first node. In fact, the trace behaves like the main bundle of a separate stalk running upwards from sucker to node. It is easy to construct stages of development in which such a stalk might become united with the main axis. *Avena* would then represent the epoch when external union was accomplished, but the vascular systems of the two members were still distinct.

In the *Zea* type the process of fusion has gone further. The scutellum trace runs upwards within the stele, but can still be distinguished from the other constituents with more or less certainty.

C. *Triticum* type.

The third anatomical type of Grass seedling distinguished by Van Tieghem possesses no mesocotyl. The apparent insertion of the scutellum takes place at the first node. We have now to examine the relation of this type to the others, assuming that some such relation can be established.

*Triticum vulgare*, Vill. The external features of the seedling are shown in Text-figs. 25-7, p. 200. The leaf within the coleoptile becomes the first foliaceous leaf. Two cauline roots are formed very early, and soon equal the primary root in length.

The scutellum is rounded at the apex. It contains slender vascular bundles, which collect lower down into a single bundle-trunk. The epithelium is confined to its dorsal surface, merely tipping the apex and fringing the ventral wings. The bundles either terminate under these fringed margins, or in the neighbourhood of the dorsal epithelium. At a level rather nearer the insertion than the apex, a main trunk is formed by union of all the upper bundles. They approach along lines converging from both margins and from the dorsal surface; their bases in transverse section look rather like the sticks of a fan.

The main bundle-trunk when first formed is much extended laterally. Its xylem is a thin ventral plate, bisected by a few protoxylem elements. Its phloem is shaped in transverse section like a flat plano-convex lens. Just below the apparent insertion of the scutellum, however, the bundle becomes more compact.

The real insertion of the scutellum on the axis can be followed with accuracy in those seedlings only where the plumule lies in a straight line with the primary root, and is fairly parallel to the midrib of the scutellum.

In such specimens the plumular traces, the stele of the primary root, and the main scutellum bundle are all cut transversely in the same series of sections. In many seedlings, however, the base of the plumule makes a considerable angle with the scutellum and primary root, or is almost perpendicular to them. Such specimens are useless for interpretation of the nodal structure. We are fortunate, however, in having secured series of sections through four straight seedlings, two in which the plumule is still enclosed in the coleoptile, and two rather older—the first leaf having pushed its way

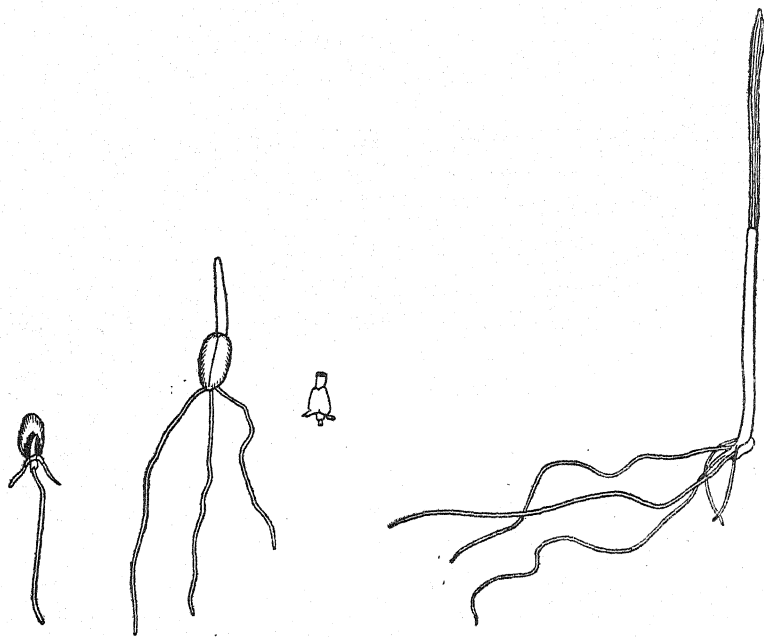


Fig. 25.

Fig. 26.

Fig. 27.

TEXT-FIGS. 25-7. *Triticum vulgare*, Vill. 25. Outline of very young seedling, life-size. 26. Outline of older seedling, life-size. 27. Outline of still older seedling, life-size.

out. The following description of insertion and nodal structure is founded on those four seedlings only.

In the two younger seedlings the main bundle of the scutellum runs straight into the stele on entering the axis. But in the two older specimens it turns upwards, and is parallel to the stele for a short distance before entering it. Thus a few sections show the inverted scutellum trace side by side with the stele, just as in *Avena*. A mesocotyl can hardly be said to exist, for the vertical distance during which the inverted scutellum trace is distinct from the stele is very small. In our best specimen it does not exceed 0.05 mm. The structure of the node is certainly clearer for this brief interval, however, and more readily comparable with that of *Avena*.

Thus the first node can be distinguished from the entry of the scutellum bundle into the axis, although in the younger seedlings both occur together. For clearness we begin with one of the older seedlings in which the series is fairly complete and the preparations successful. The other is less clear, but the main points can be verified in it by comparison with the first.

After remaining distinct from the stele through a few sections,<sup>1</sup> the phloem of the upward scutellum trace divides to right and left of the xylem. One half goes to form the coleoptile trace *P*, the other to form *P'*. The xylem meanwhile splits into three parts; the lateral branches accompany the phloem, while the median elements enter the stele at the gap which faces the midrib trace *M* (cf. II and III, Text-fig. 28, p. 202). They turn downwards at once, and do not, therefore, appear in Diagram I of Text-fig. 28.

So much for the behaviour of the scutellum trace on its upward course. A more complete view of the first node is gained by following the coleoptile and plumular traces downwards.

A little distance above the first node are eight well-marked plumular traces, besides several small ones. Seven of these belong to the first leaf. The eighth is the midrib of the second leaf, which divides just above the entry of the coleoptile bundles and inserts itself on two lateral traces. The coleoptile bundles enter by the gap thus left, which is opposite *M*, the midrib trace from the first leaf.

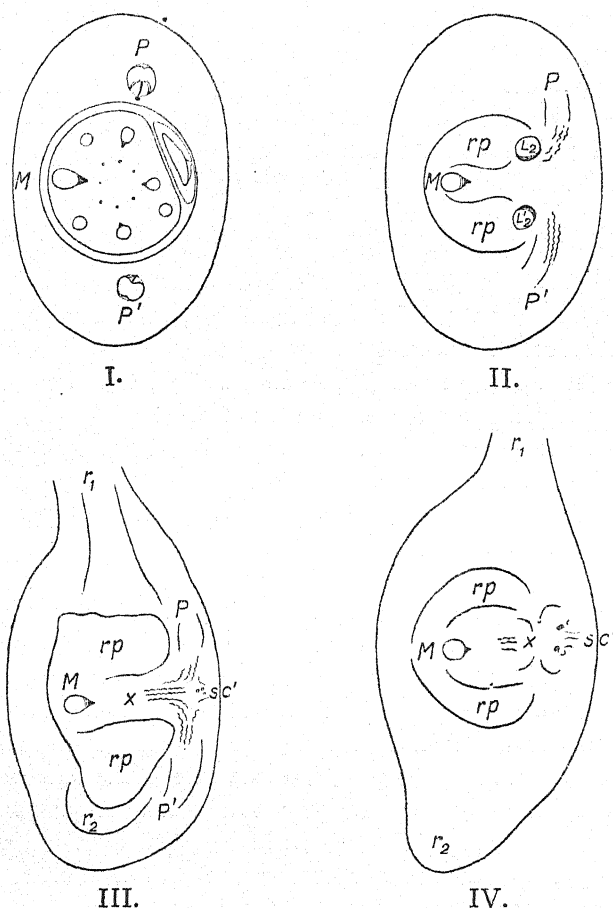
The terminations of the coleoptile bundles are sharply bent, one lying over the other, but quite distinct. They lie just under the apex of the coleoptile, and on the same side of it. A number of spiral tracheides are found at the angle of each. Their function is doubtless related to the occurrence of the large water-pores which are always found in their neighbourhood. Near the first node the bundles are not clearly double in transverse section, and they lie a little to one side of the line which bisects stele and axis symmetrically (I, Text-fig. 28). Nor do they meet within the stele as in *Avena*, but just outside it (II and III, Text-fig. 28, p. 202). The branching at this spot is, however, precisely that of *Avena*. The ingoing branches—a common xylem strand and two distinct phloem groups—enter the stele by the gap opposite *M*, and constitute the bundle *x*. The outgoing branches unite to form the inverted scutellum trace, *sc*. There is also a direct xylem connexion—as explained above—between the scutellum trace and *x*. In this respect again *Triticum* resembles *Avena*.

This vascular symmetry may be described as essentially that of an *Avena* seedling in which the mesocotyl has been reduced in length almost to vanishing point. In the two younger *Triticum* seedlings it

<sup>1</sup> The sections are cut to a uniform thickness of about 10  $\mu$ .

has disappeared altogether, and the insertion of the scutellum bundle takes place at the first node.

The process may be shortly described from above downwards. The coleoptile bundles meet outside the stele, but the external branches go to form the main bundle of the scutellum itself, not merely an inverted trace.



TEXT-FIG. 28. *Triticum vulgare*, Vill. Four diagrams of axis in transverse section. I. Base of first internode; bud in axil of coleoptile. II. Top of first node; *rp*, *rp*, root-plates. III. Middle of first node. IV. Base of first node; *sc''*, scutellum trace.

The internal branches form a compact bundle which is prolonged downwards below insertion level, and can be recognized until the stele becomes root-like. There is a direct connexion by xylem arch between this trace and the scutellum bundle. In both the seedlings we are now considering the cauline roots are rudimentary, but the root-plates are present below the first node, and effectually mask the transition to root-structure. In the

younger of the two seedlings very little tissue is lignified in this region, and this adds to the obscurity in structure.

We have already remarked that the chief difference in vascular symmetry between the seedlings of *Avena* and *Triticum*, besides the absence of a mesocotyl in the latter, lies in the insertion of the coleoptile bundles. In *Avena* each enters between two lateral traces from the first leaf, and unites with both. The root-plates depend chiefly on traces from the second and third leaves. In *Triticum* the lateral traces of the first leaf go bodily into the root-plates, and the coleoptile traces enter the stele in the by-gap between those plates. The difference can be appreciated most easily comparing Diagram II in Text-fig. 16, p. 174, with II in Text-fig. 28. The younger seedlings of *Triticum*, in which the root-plates are not differentiated at that level, show the insertion of one lateral leaf-trace on either coleoptile bundle.

This anatomical distinction is no doubt correlated with the single root-system of the *Triticum* seedling. In *Avena* the first leaf is supplied with water through the mesocotyl from the lower root-system, which includes the lower set of cauline roots as well as the primary root. The first leaf is therefore mainly connected with the traces which run down the mesocotyl. But in *Triticum* there is only one system of cauline roots. They correspond to the upper or nodal root-system of *Avena*. While the first leaf draws water from the primary root by its midrib, it must also be in direct connexion with the nodal roots through their insertion-plates.

*Hordeum vulgare*, L. In external characters the seedling resembles that of *Triticum*, but the cauline roots are more numerous. In all the seedlings examined anatomically, the first leaf is still enclosed in the coleoptile.

The scutellum has two main bundles symmetrically placed. Each resembles the single bundle of *Triticum* in being a trunk, built up of the slender branches which ramify in the apical region of the scutellum. When first formed, both trunks are nearly or quite amphivasal: lower down, the xylem in each becomes a ventral crescent. Just above their insertion on the axis the xylem groups are much extended laterally, and each is bisected by a group of protoxylem elements.

The dual symmetry of the scutellum simplifies the ground-plan of its insertion. For when the two bundles run into the axis, the phloem of each turns outwards, and passes into the adjacent coleoptile trace. The greater part of the xylem of each bundle accompanies the phloem, but the inner xylem elements are in contact with each other, and go straight to the stele, where they meet other elements from the incoming coleoptile bundles. All this xylem turns downwards, and forms a massive strand within the stele for a short distance below the node. It is soon lost in the tangled anatomy of root-insertions.

These changes in structure are more readily compared with those recorded in *Triticum* and *Avena* when they are described in the inverse order, that is, from above downwards.

Seven traces from the first leaf and two bundles from the coleoptile enter the first node from above. This does not include the midrib trace *m* from the second leaf, which is commonly present at the beginning of the first node. But as it splits into two branches which unite with two lateral traces from the first leaf before the first node ends, it is not considered as having a course distinct from theirs. Some traces—usually unligified at this age—which represent the lateral bundles of the second leaf, also enter the first node; and in the older seedlings examined, certain strands on either side of *m* can be traced back to the bud in the axil of the coleoptile. Sooner or later all these traces are absorbed in the root-plates (I and II, Text-fig. 29).

The midrib trace *M* from the first leaf remains distinct throughout the node, and is always opposite the gap left by the defection of *m*. Lower down this gap is filled by the temporary union of the two coleoptile traces *P* and *P'*; and a branch from each trace turns downwards into the stele while they are still in contact. Their phloem groups are distinct at first, but soon form a crescent external to the common xylem group, which contains, besides the two xylem branches from the coleoptile traces, some central elements from the scutellum bundles (III, Text-fig. 29).

Thus, at the base of the first node, we find within the stele a single bundle *M* facing a compound bundle *x*, built up of branches from *P* and *P'*, together with xylem elements from the scutellum traces. These bundles *M* and *x* are separated from each other on either side by a plate of xylem and phloem (*xp*, *xp'*), derived from the lateral traces of the first and second leaves (IV in Text-fig. 29). This very definite structure is completely obliterated a little lower down by the insertion of four cauline roots, and when the confusion introduced by them has disappeared, the main stele of the axis is found to be root-like.

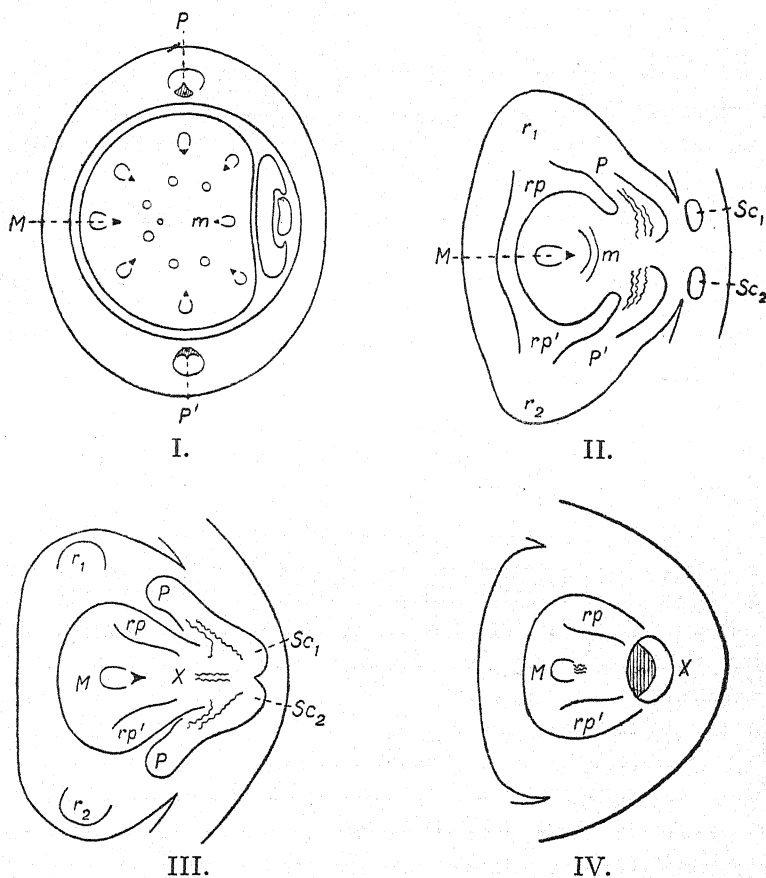
This accounts for the internal branch from each coleoptile trace. But the larger portion of each trace pursues its course through the cortex of the axis, and enters the scutellum. Turning sharply upwards, each coleoptile segment becomes one of the two bundle-trunks described above (*sc*<sub>1</sub>, *sc*<sub>2</sub>, II and III, Text-fig. 29).

The *Hordeum* seedling differs from that of *Triticum* chiefly in two points: in the presence of two scutellum bundles, and in the absence of any approach to a mesocotyl. We might have attributed the second character to the youth of the *Hordeum* seedlings examined, but by the kindness of Mrs. Taylor we have been able to compare our series with another cut by her from an older specimen. This corresponds in age with the older



*Triticum* seedlings described by us. As in our younger specimens, the scutellum bundles do not turn upwards in the axis before joining the stele, but enter the coleoptile traces at once.

The cultivated species of *Hordeum* are said by Van Tieghem to have two bundles in the scutellum; the wild species to have but one. We have verified this in the wild species *H. jubatum*, L. Two interpretations of this



TEXT-FIG. 29. *Hordeum vulgare*, L. Four diagrams of axis in transverse section. I. Base of first internode; bud in axil of coleoptile. II. Top of first node and apparent insertion of scutellum;  $sc_1$ ,  $sc_2$ , scutellum bundles. III. Middle of first node and real insertion of scutellum. IV. Just below insertion of scutellum.

distinction are possible. The cultivated varieties may be derived from an ancestral form with two bundles, now extinct. We might then regard the dual symmetry of the scutellum as a primitive character. Or the ancestral scutellum may have had but one bundle as in the wild species which survive. In that case the doubling of the bundle in cultivated forms is probably adaptive, a response to the demand for more vascular tissue. The

ready splitting of the bundle-trunk to correspond with the twofold structure of the coleoptile might indeed suggest reversion to an originally dual symmetry. Otherwise the increase in mass of vascular tissue might perhaps have been effected by the division of the bundle-trunk into three or four; or by the more vigorous branching of a single trunk. We cannot, however, attach much weight to this argument, considering our ignorance of the laws of adaptation.

*Comparison of the Triticum type with the Avena type.*

The vascular symmetry of the *Triticum* seedling is easily derived from that of *Avena* by suppression of the mesocotyl. And this suppression is not merely hypothetical, for in certain *Triticum* seedlings we have found a very short mesocotyl, in which the anatomy is precisely comparable to that of *Avena* (*ante*, p. 200). We have not made any similar discovery in *Hordeum vulgare*, where the mesocotyl seems always absent. In other respects its vascular symmetry is that of *Triticum*, substituting two bundles for one in the scutellum. We have already discussed the significance of this character.

## II. THE ANATOMY OF CERTAIN OTHER MONOCOTYLEDONOUS SEEDLINGS COMPARED WITH THAT OF THE GRASSES.

The three types of seedling structure distinguished by Van Tieghem in the Gramineae appear at first sight astonishingly different. The absence of a mesocotyl distinguishes the *Triticum* type from the others, and modifies its anatomy profoundly. The insertion of the scutellum trace on the stele occurs at the base of the hypocotyl in *Zea*, while it is postponed to the first node in *Avena*. On the other hand, certain anatomical features found in all the types make comparison with other Monocotyledons difficult. For example, the two opposite and equivalent bundles in the coleoptile distinguish it from the scutellum, with its single bundle on the one hand, and from the plumular leaves on the other. It cannot be satisfactorily treated either as the cotyledon or as the first leaf. Again, the double structure of the coleoptile bundles—particularly plain in *Avena*, *Sorghum*, and *Zea*, and very clearly indicated in *Triticum*—can hardly depend on the double character of the scutellum trace, since only one-half of the latter reaches each coleoptile bundle. Finally, in all the types the coleoptile bundles appear to arise at the first node, partly from the scutellum trace, and partly from the stele of the mesocotyl.

These features are explicable if all three types are derived from an imaginary ancestor *X*, with the seedling skeleton suggested in Text-fig. 7, p. 166. We have already considered the modifications of structure necessary to convert that ancestor into a seedling of the *Avena* type (pp. 168–9). None of

them are improbable. Fusion between two distinct and adjacent members occurs very commonly in all parts of the plant ; there is no morphological reason why the stalk of the cotyledon should not unite with the hypocotyl to form the mesocotyl. More or less complete fusion of two separate bundles within the cotyledon is frequent among Monocotyledons, and may be supposed to occur in the sucker and stalk of type *X*. When these two changes have been accomplished, the sucker of the cotyledon will appear sessile on the axis at the base of the mesocotyl, while its trace runs upwards to the first node. In *Avena* the inversion of this trace, and its double character, suggest its origin from the two bundles of the stalk in a form such as *X*.

A third modification is necessary to convert the sheath of type *X* into the coleoptile of the *Avena* type. The double scutellum trace, representing the two stalk bundles of *X*, divides at the node, and if each half-trace behaved as in *X*, it would run nearly to the top of the sheath, and turning sharply down again join the mesocotylar stele at the first node. The more acute the angle made by the half-trace on itself, the nearer would the ascending and descending segments lie to each other. If they approached so closely as finally to unite from first node to apex, the coleoptile bundles of *Avena* would be reproduced. That such fusion between two lengths of the same bundle may actually occur is shown in the sheath of *Tigridia* (Text-fig. 8, p. 168, and Pl. X, Fig. 15).

The two sheath-bundles of type *X* finally enter the stele of the hypocotyl from opposite sides, and presumably remain distinct within it. In *Avena* the descending segments of the coleoptile bundles unite in the stele of the mesocotyl—the fourth modification required to convert type *X* into the *Avena* type. But in the *Zea* type, best represented in this respect by *Coix*, no such alteration in structure is demanded. The coleoptile half-traces remain distinct in the stele ; separated from each other by the ascending scutellum trace, which in this type is included within it.

We have implied throughout this discussion that the *Avena* type is the most primitive of the three described by Van Tieghem. Indeed, this conclusion follows from our conception of the mesocotyl. For if it represents the hypocotyl of a remote ancestor united with the stalk of its cotyledon, the two types possessing a mesocotyl are nearer to that ancestor than the third which has none. And in the *Avena* type the ancestral stalk is represented by a separate trace, which in the *Zea* type is absorbed in the stele of the hypocotyl. Thus on our hypothesis the vascular skeleton of the *Avena* seedling represents the ancestral type *X* more completely than that of *Zea* or *Sorghum*.

But though, on the whole, the seedling skeleton of *Avena sativa* has more points which suggest the imaginary type *X* than that of any other species we have examined, yet certain isolated characters are better repre-

sented elsewhere. Thus the formation of the scutellum trace from the coleoptile bundles is better shown in *Zizania aquatica*, their double structure in *Sorghum vulgare*, and the coleoptile traces within the stele retain their identity longer in *Coix Lacryma-Jobi*.

Nor is it strictly accurate to say that the *Triticum* type can be derived from the *Avena* type in one way, and the *Zea* type in another. Our hypothesis is better formulated thus: the seedling skeleton of the common ancestor from which are descended all the genera we have examined probably resembled the *Avena* type in the possession of a mesocotyl, and in the less complete union of the two members from which it is derived. A greater number of the structural variations depending on these characters are found in the *Avena* type than in the others, and it may be called primitive with regard to them, since it approaches more nearly to our conception of an ancestral form within the Gramineae.

This ancestral form, which may be referred to as *G*, is not to be confused with type *X* figured in Text-fig. 7, p. 166. *X* has none of the anatomical features characteristic of the Gramineae, but illustrates a skeleton from which such features might be readily derived on the one hand, while on the other it has the seedling characters of a hypogeal Monocotyledon.

We shall now describe the seedling structure of certain Monocotyledons which approach more or less closely to the imaginary type *X*, in order to justify our use of that construction in explaining the anatomy of the Grass seedling. Before entering on this subject, however, we must explain the absence of any reference to the Cyperaceae. We naturally expected that the seedling structure of this family would throw light on that of the Grasses. But the vascular skeleton of the species we have examined is too much reduced to be significant. Even the seedlings of the comparatively robust *Cyperus alternifolius*, L., and *C. natalensis*, Hochst., are as small and tender as those of an aquatic plant.

We have already stated that Van Tieghem considered the mesocotyl to represent an elongated node, whereas we look on it as a fusion of the hypocotyl with part of the cotyledon. On either hypothesis the coleoptile of the Grass seedling is identified with the upper or stipular sheath.

An upper sheath is not universal even among hypogeal Monocotyledons, and cases in which it contains vascular tissue are comparatively rare outside the Glumiflorae. Such cases are, therefore, likely to be instructive, and we have already referred to some of them. We propose to discuss these and others in greater detail, beginning with the seedlings of *Elettaria Cardamomum* and its allies.

## Zingiberaceae.

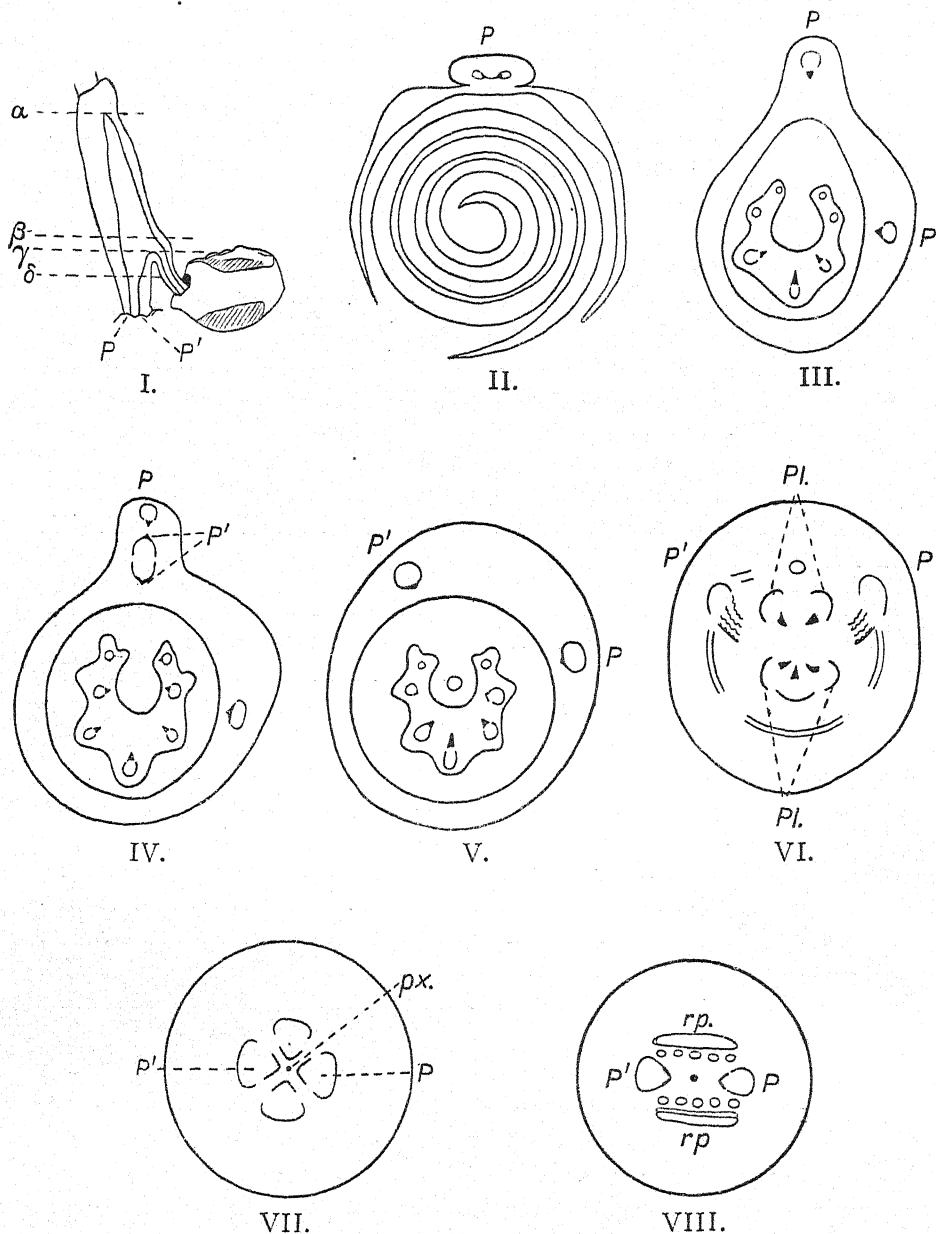
Seedlings in spirit belonging to three genera from this family were sent from Kew in 1898 to one of us: *Elettaria Cardamomum*, Maton, *Amomum angustifolium*, Sonner, and *Renealmia racemosa*, A. Rich. Preparations from these species were made by Miss E. N. Thomas in the Reigate laboratory, and she also worked out the course of the bundles in the upper sheath, which is uniform in all three. Our thanks are due to her for permission to use her preparations and notes, including some drawings of the seedlings. We have since confirmed and extended her observations by cutting fresh seedlings of *Elettaria*, and also by examining *Roscoea purpurea*, Sm., *Alpinia calcarata*, Rosc., and *Brachychilum Horsfieldii*, O. G. Petersen. These seedlings we grew in a hothouse at Reigate, from seeds most kindly supplied by Mr. Lynch from the Botanic Garden at Cambridge. Thus we were able to examine and draw them in the fresh condition, besides pickling the important parts, the sheath and hypocotyl, in Merkel's solution. Material so treated gives far better results when microtomed than that preserved in spirit only.

*Elettaria Cardamomum*, Maton. The course of the bundles in the cotyledon is very characteristic. Text-fig. 30 (I), p. 210, is a drawing of stalk and sheath from spirit material examined under the simple microscope. The course of the bundles in the sheath can be quite well followed by this method, and they are traced in the drawing. It will be seen at once that part of the sheath is above the insertion of the stalk, and part below it.

The stalk of the cotyledon contains two exarch collateral bundles, both of which enter the sheath. Their course within it is asymmetrical, for one bundle (*P*) travels nearly to the top of the sheath before turning downwards, while the other (*P'*) turns down at once. We possess a complete series downwards, from the top of the same sheath which is drawn in I (Text-fig. 30), and can therefore reconstruct its vascular skeleton with certainty. The critical sections (II-V in Text-fig. 30) occur at the levels  $\alpha$ - $\delta$  in I.

Sections cut between the levels  $\alpha$  and  $\beta$  pass through bundle *P* twice; once in its upward, and once in its downward course. Near  $\alpha$  the two sections of this bundle lie close together, while lower down they move apart until they are separated by an angle of about  $90^\circ$ . Just below  $\beta$ , the bundle *P'* comes in, and in the following sections *P* and *P'* are each cut twice: once in the sheath, and once in the stalk. From  $\delta$  onwards the stalk has disappeared, and then the two sheath-sections only of *P* and *P'* remain (V, Text-fig. 30).

*Elettaria* possesses a real hypocotyl; a region of appreciable length below the first node, in which the stele is stem-like. The hypocotyl varies



TEXT-FIG. 30. *Elettaria Cardamomum*, Maton. Structure of sheath and hypocotyl. I. Seed and sheath enlarged, showing course of cotyledonary bundles. II. Diagram of sheath at level  $\alpha$ . III. Diagram of sheath at level  $\beta$ . IV. Diagram of sheath at level  $\gamma$ . V. Diagram of sheath at level  $\delta$ . VI. Diagram of first node. VII. Diagram of hypocotyl (top). VIII. Diagram of hypocotyl (base).

in length from 0.8 mm. to about 1.5 mm. in the three seedlings from which we have fairly complete series.

In seedlings of the age shown in Text-fig. 30, the first leaf has seven traces. The five larger run inwards at the second node, but each gives off one or two slender downward spurs before doing so. Hence a circle of seven or eight strands outside the stele of the first internode, in which the gap opposite the midrib is filled by the two marginal traces from the first leaf. These are still in their original position near the periphery of the section. They may unite to form a larger trace.

The traces in the stele just above the first node are reduced to five, arranged in two groups. The cotyledonary traces run in from either side between these two groups. But as they do so, each strand or trace in the outer circle extends itself tangentially, in such a way that the two cotyledonary traces are included in a vascular girdle which is concentric with the stele, but quite distinct from it. This girdle is shown in process of formation in VI, Text-fig. 30. In the *Elettaria* seedling on whose structure these diagrams are founded, the xylem of the girdle is unligified except at one point, but we have found a girdle much better differentiated in *Amomum* (Pl. X, Fig. 13), and here the xylem is very well represented. The function of this girdle is probably to give rise to cauline roots, and its presence may indicate the approaching formation of a tuber.

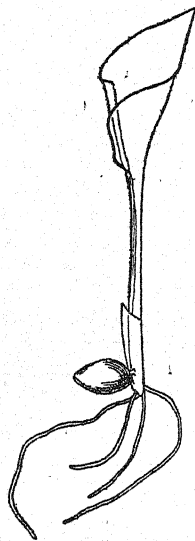
At the top of the hypocotyl, as soon as all traces of the first node have disappeared, there are four massive phloem groups, each very distinctly triangular in transverse section. Two of these are cotyledonary, and they are diagonally opposite to each other. The two groups which separate them are derived from the plumule. The arrangement of xylem is characteristic. All the protoxylem elements form a little group in the centre of the stele. The internal angles of the four triangular phloem groups are bordered by metaxylem for some distance up each side. In the oldest seedling examined, the xylem has the form of a four-rayed star. Each ray is double, for it consists of metaxylem border from each of two adjacent phloem groups, separated by a strip of medullary tissue. Even in this seedling it is clear that the metaxylem bordering the plumular phloem is formed of elements larger and better lignified than that which borders the cotyledonary groups. This difference is more clearly marked in the younger seedlings, where much cotyledonary xylem is still unligified.

Lower down, the symmetry of the stele alters, to provide for the insertion of cauline roots. The double rays become single by suppression of the medullary intervals, and then two parallel root-plates are formed from them. These root-plates are divided from each other by the cotyledonary bundles, which retain little or no metaxylem, but are each represented by a triangular patch of phloem and a small internal group of xylem elements.

The two groups of plumular phloem are extended in strips, each external to a xylem plate (VIII, Text-fig. 30).

At a level very little below the formation of root-plates, the stele of the axis is completely masked by the repeated insertion of cauline roots. We could not determine in the descending series whether any one of the roots, all cut more or less obliquely, represented the primary root. It may perhaps never develop at all; its functions being taken over at once by the cauline roots.

*Amomum angustifolium*, Sonner. Two seedlings were examined, both about the same age. That drawn by Miss Thomas in Text-fig. 31 was cut



TEXT-FIG. 31. *Amomum angustifolium*, Sonner. Outline of seedling, slightly enlarged.

by hand. The bundles of the cotyledonary sheath were previously traced by her under the simple microscope. From the other she cut a complete series of sections, beginning half-way down the upper sheath, and ending with the tangled insertions of cauline roots at the base of the hypocotyl.

The cotyledon of *Amomum* possesses two large bundles in its stalk; one of them runs nearly to the top of the upper sheath and then turns downwards, while the other hardly enters it. The vascular skeleton of stalk and sheath, indeed, is precisely that of *Elettaria*, and might be represented by Diagrams I-V, Text-fig. 30, with a few alterations in matters of detail.

The cotyledonary bundles  $P$  and  $P'$  enter the stele of the axis in the same way at the first node. The vascular girdle is better developed, perhaps because the seedlings are older than in *Elettaria*; and it seems as if  $P$  and  $P'$  took an active share in its formation by branching to meet the cortical traces (Pl. X, Fig. 13). At the top of the hypocotyl, the formation of a xylem star with double rays is even clearer than in *Elettaria* (Pl. X, Fig. 14); but it passes over into the single-rayed form more quickly, and this persists longer. The formation of root-plates is obscure; before they are well defined, the stele is lost among cauline root-insertions. We could not determine whether the primary root was undeveloped, or whether it existed but was hopelessly lost in the series among the sections of cauline roots.

*Renealmia racemosa*, A. Rich. Two seedlings were cut by Miss Thomas from spirit material. The series from the first is fairly complete from insertion of stalk downwards, but the xylem is very little lignified.



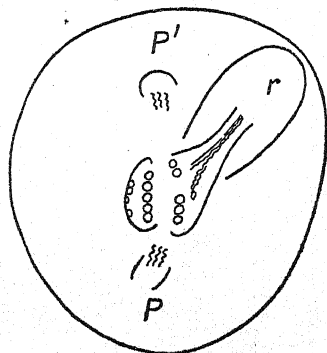
The second series is very incomplete, but serves to confirm the first, and in some cases to explain it, as the tissues are better differentiated.

The bundles of the upper sheath behave as in *Elettaria* and *Amomum*. The lower sheath is comparatively short. The cotyledonary traces *P* and *P'* enter the stele of the hypocotyl as usual from either side. In this species the plumular traces seem to form three groups in place of two. In both the seedlings examined there were five traces at the top of the hypocotyledonary stele; in one of them the fivefold symmetry was retained throughout, in the other it became fourfold lower down. The protoxylem forms a single group in the centre. No well-marked root-plates are formed. The cauline roots arise from the base of the hypocotyl.

*Roscoeia purpurea*, Sm. The cotyledon in this species differs from that of *Elettaria* in the structure of its sheath. Of the three seedlings examined by us, we find two with no lower sheath, and the third with a very short one. The upper sheath is very well developed in all three seedlings, and its vascular skeleton corresponds exactly with that of the upper sheath in *Elettaria*.

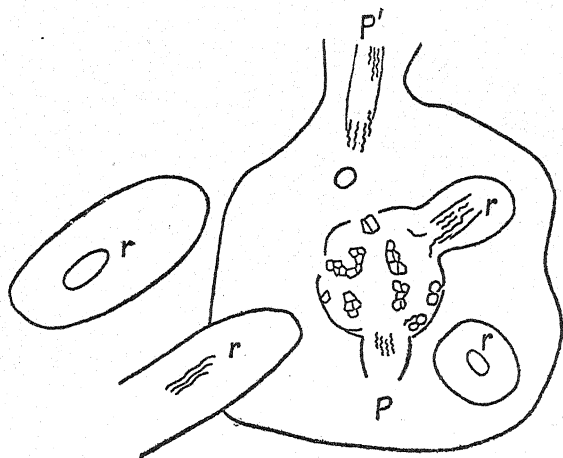
The vascular structure of the hypocotyl and first node differ in the two genera, but the points of difference are clearly correlated with the presence or absence of a lower sheath. Thus in that seedling of *Roscoeia* which has a lower sheath, though a very short one, and is therefore nearest to the *Elettaria* type, there are about a hundred sections in which the upper sheath corresponds in structure to Diagram III in Text-fig. 30, except that in the lower ones the bundles are separated more widely. But whereas in *Elettaria* only the first leaf is enclosed by this region of the sheath, in *Roscoeia* we find sections of the plumular axis. The length enclosed is about 1 mm.; it begins at the base of the plumular bud, and ends in the first internode. The single sheath section which corresponds to IV, and the few below it which represent V, still enclose sections of the first internode. In *Roscoeia* cauline roots are formed above the first node, and they penetrate the sheath in region V.

Thus the cotyledonary traces *P* and *P'* run into a stele which is already giving off cauline roots, and has formed root-plates. In place of Diagram VI in Text-fig. 30 we have Text-fig. 32. There is no level in this seedling which corresponds to VII. In the intervals between the insertion of cauline roots, the stele of the hypocotyl resembles VIII in structure, though the



orientation is different. For in *Elettaria* the bundle  $P'$  travelled through an angular distance of about  $90^\circ$  in the sheath before running into the stele. Taking the insertion of the stalk on the sheath as a fixed point, and making the diameter of the section which passes through it vertical, the cotyledonary traces  $P$  and  $P'$  in VIII lie on the horizontal diameter. But in *Roscoea* the trace  $P'$  runs straight into the stele from the stalk, and  $P$  travels round to the opposite extremity of the vertical diameter before entering the stele.

In the two other *Roscoea* seedlings there is no lower sheath at all, which leads to greater modification in the vascular skeleton. The insertion of the upper sheath coincides with level IV, and the section of  $P'$  which appears in



TEXT-FIG. 33. *Roscoea purpurea*, Sm. Diagram of first node in two other seedlings.

the sheath is already running into the stele of the axis (Text-fig. 33). Root-plates are formed early, to correspond with the great development of cauline roots at the node ( $r, r, r, r$ ). In one seedling the plates are found above the node; in the other they begin just below it. Between root-insertions the root-plates sometimes break up. Sections can be found which suggest the symmetrical arrangement of VII in the *Elettaria* diagrams.

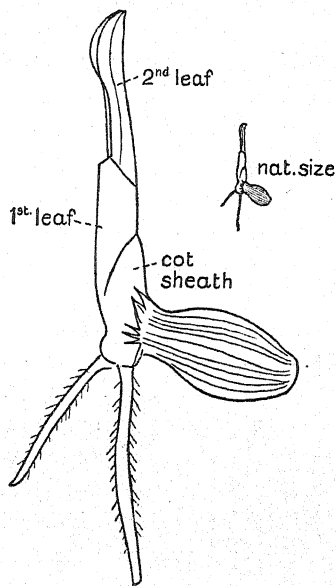
Though we have used Diagrams VII and VIII from *Elettaria* to explain the structure of *Roscoea*, there is one great difference between the two genera. In all three *Roscoea* seedlings the central group of protoxylem is absent. Each root-plate and each cotyledonary trace retains its own protoxylem, which is more or less definitely internal to the other elements.

*Alpinia calcarata*, Rosc. We have made preparations from four seedlings, and drawings of five. They are small compared with those of *Elettaria*. The cotyledonary sheath is short, and the plumule very soon

emerges from it. The first foliage leaf of the plant is the second in order on the axis, for the first is reduced to a mere sheath.

Two distinct bundles enter the stalk of the cotyledon from the sucker.<sup>1</sup> The stalk is inserted rather above than below the junction of sheath with axis. There is no true lower sheath. In three seedlings the upper bundle *P* runs a little way into the upper sheath before it turns down to join the axis. In the remaining seedling, the youngest, *P* runs up from the stalk, and continues its course through the sheath into the axis. The second bundle, *P'*, commonly enters the axis from the stalk below the apparent insertion. Since *P* and *P'* enter the stele from opposite sides, one or other of them must skirt it for some distance before turning in.

The stele of the hypocotyl is cut so obliquely in the three older seedlings, that its structure cannot be deciphered. In the youngest seedling, the traces settle down to three endarch bundles, which seem to correspond in position to the midrib and lateral bundles of the second leaf. They are separated by three rays of medullary tissue, forming angles of about  $120^\circ$  with each other. *P* enters by one of the two rays which border the midrib of the second leaf, and *P'* by the other. The third ray remains clear. Below this level the stele of this seedling too is bent, and the sections of it hopelessly oblique. Apparently there would be five bundles at the top of the hypocotyl, as in *Renealmia*.



TEXT-FIG. 34. *Brachychilum Horsfieldii*, O. G. Petersen. Outlines of seedling, life-size and enlarged.

*Brachychilum Horsfieldii*, O. G. Petersen. Preparations were made from five seedlings by one of us, including complete series through the cotyledonary sheath in four of them. The cotyledon is inserted partly on the sheath and partly on the axis as in *Alpinia*. The first leaf is reduced to a mere sheath (Text-fig. 34).

There are two distinct bundles in the apex of the cotyledon, and they run side by side through the stalk into the axis. In three seedlings neither bundle enters the sheath at all; in the fourth, one trace (*P*) lies rather above the other, and it curves upwards into the base of the sheath, just entering it before turning back into the axis. The short upper sheath,

<sup>1</sup> For apparently mesarch structure of stalk-bundles in *Alpinia*, *Brachychilum*, and *Roscoea*, see E. M. Berridge in Ann. of Bot., vol. xxiv, p. 485, 1910.

indeed, may be said to be without vascular tissue even in this case. In the others,  $P$  and  $P'$  alike run upwards from the stalk of the cotyledon into the stele, diverging as they penetrate the axis in order to enter the stele at different points.

In two seedlings the stele of the hypocotyl is cut transversely where  $P$  and  $P'$  enter it. In both cases the three plumular bundles correspond in position to the principal traces of the second leaf. The cotyledonary traces enter on either side of the midrib. Thus there are five traces at the top of the hypocotyl, but they cannot be followed downwards in any series of preparations which we possess, partly because the hypocotyl is always curved, and partly on account of the numerous root-insertions.

*The seedling structure of the Zingiberaceae.* We have already compared the sheath of *Elettaria* with the coleoptile of *Avena*, and have constructed an imaginary form with a vascular skeleton intermediate between the two (Text-figs. 5-7, p. 166). The force of this comparison is much increased by the observations just recorded on five other genera of the Zingiberaceae. For characters common to several genera within a family, and—so far as we know—confined to that group, are probably ancient. The presumption is that they are inherited from a common ancestor.

All six representatives of their genera have two distinct bundles in their cotyledon; in four genera these bundles enter the upper sheath. Their course within it is alike in all four, and very characteristic. In other words the vascular skeleton of the sheath in *Amomum*, *Renealmia*, and *Roscoea* is almost identical with that of *Elettaria*, and is different from the skeletons of any other cotyledonary sheaths which we have examined. In *Alpinia* the upper bundle from the cotyledon sometimes enters the sheath, but only penetrates it for a very short distance before turning back. Even in this reduced form the same asymmetric type of skeleton can be recognized. In *Brachychilum* the short upper sheath contains no bundles.

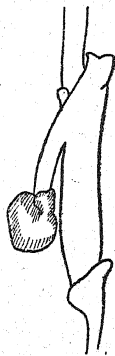
These genera have other anatomical characters in common. The first node is alike in all of them. The cotyledonary traces enter the stele of the axis from opposite sides, making an angle of at least  $120^\circ$  with each other. They take their place among the two or three plumular traces which are continued downwards. In all the species examined, except *Roscoea purpurea*, a common group of protoxylem is found in the centre of the hypocotyledonary stele, which recalls the common group formed by the junction of  $P$ ,  $P'$ , and  $M$  in the mesocotylar stele of *Coix*. This afterwards breaks up into the groups  $px_1$  and  $px_3$  (p. 198).

Cauline roots are given off freely in the neighbourhood of the first node, and sometimes lower down. The well-defined root-plates found in the hypocotyl of *Elettaria* (Text-fig. 30, VIII), *Amomum*, and *Roscoea* recall the root-plates in the mesocotyl of *Avena* and other Grasses.

The upper sheath in the Zingiberaceae, when it contains any vascular tissue at all, is stiffened by one of the bundles which enter it from the stalk of the cotyledon. After running obliquely upwards, this bundle turns sharply down, and enters the axis from the base of the sheath. No part of such a bundle passes directly from stalk to axis. The second bundle may do so (*Roscoea*, *Alpinia*), or it may just enter the upper sheath on the side opposite the first bundle, and then turn downwards through the lower sheath to the axis (*Elettaria*, *Amomum*, *Renealmia*). But all the bundles found in the upper sheath enter it from the stalk of the cotyledon, and leave it on their way to the hypocotyl. The same is true of *Tigridia* (Text-fig. 8, p. 168) and of *Commelina coelestis*, which we are about to describe. But this form of vascular skeleton is not universal in the upper sheath of Monocotyledons. We shall describe the seedling of *Colchicum autumnale* as an example of another type.

*Commelina coelestis*, Willd. The lower sheath is long, and consists of the cylindrical base of the cotyledon enclosing the plumule. Above it is the upper sheath, which forms a hood and seems to have arisen from a sharp twist in the stalk of the cotyledon, just where it was spreading out into a simple sheathing base (Text-fig. 35). Two main bundles enter the sheath from the stalk, and behave very much like those of *Elettaria*. The upper bundle (*P*) travels nearly to the top of the hood before bending back to the axis, while the lower one (*P'*) turns down almost as soon as it enters the sheath.<sup>1</sup> One or two additional bundles are sometimes found in sheath or stalk, but they are slender and end blindly, and are probably mere mechanical stiffenings, produced where they are needed.

In *Commelina* the asymmetrical course of the two main bundles is probably due to the distortion of the originally simple sheath. After they have traversed the lower sheath, they approach the stele of the axis from opposite sides as in *Elettaria*. Two plumular traces are present at the first node, but there the resemblance ends. The plumular traces insert themselves on *P* and *P'*, each of which becomes double. These double bundles face each other throughout the rather long hypocotyl, and ultimately form a tetrarch root. The anatomy of the hypocotyl is precisely that of some Dicotyledons, as for example *Althea*.<sup>2</sup> The details of transition to a root-structure are masked by the insertion of cauline roots.



TEXT-FIG. 35.  
*Commelina coelestis*,  
Willd. Outline of  
sheath and adjacent  
parts; enlarged.

<sup>1</sup> Attention has been drawn to the asymmetrical behaviour of the two bundles in the cotyledon sheath of another species of this genus by Martha H. Hollinshead: Notes on the Seedling of *Commelina communis*, L. Contributions from the Botanical Laboratory of the University of Pennsylvania, vol. iii, No. 3, p. 275, 1911.

<sup>2</sup> Gérard, R.: Ann. des sci. nat., sér. vi, Bot., t. xi, Pl. XVI, Fig. 23, 1881.

*Colchicum autumnale*, L., is an example of a hypogeal Monocotyledon in which the very well developed upper sheath is stiffened by branches from the cotyledonary bundles, and not by the bundles themselves. We have examined two seedlings of this species. The upper sheath is stiff, long, and sharply pointed. The lower sheath is cylindrical and of some length. The primary root is stout and long; cauline roots do not appear early.

We have complete series of sections from both seedlings, beginning in the upper sheath, and passing downwards, through the insertion of the stalk and the lower sheath, to the junction of sheath and axis, and the formation of the primary root. The only anatomical difference between the two seedlings is that in one of them there are two distinct bundles in the stalk of the cotyledon, each surrounded by its own endodermis, and that in the other the two stalk-bundles are in contact, and surrounded by a common endodermis. In fact, they form a typical double bundle as they enter the sheath. This difference is not so great as it may seem, for the distinct bundles of the first seedling also form a double bundle when they enter the lower sheath. In both seedlings the stalk bundles turn into it at once, and retain their characteristic double appearance until they enter the axis. But as they turn, each gives off a slender branch to the upper sheath, and these branches divide again on their upward way. They all end blindly near the apex of the sheath which they serve to stiffen.

Three plumular traces are found at the first node. They represent the midrib and two lateral traces from the first leaf. The double bundle of the cotyledon is inserted on them, but it does not affect the symmetry of the triarch root-stele. The transition to root structure is extremely rapid, and the plumular phloem groups retain their position throughout.

#### GENERAL CONCLUSIONS FROM PART II.

In the introduction to this Part (pp. 206-8) we considered how far the evidence given in Part I could be used to support our interpretation of the Grass embryo and seedling. That interpretation has been outlined at the beginning of this memoir (pp. 164-9). It is illustrated there by the construction of an imaginary type *X*, linking the *Avena* type, which we consider as the most primitive of the three distinguished by Van Tieghem in the Gramineae, with the seedling skeleton of a real hypogeal Monocotyledon, *Elettaria*.

The evidence given in Part I refers to the structure of Grass seedlings only. In discussing it, we have tried to show that all Van Tieghem's types could be derived from the imaginary skeleton *X*, without any unprecedented or even improbable changes in structure. In Part II we have described seedlings from other families, whose vascular structure approaches that of *X*, and this evidence, too, must be summed up.

The examination of monocotyledonous seedlings with a well-developed upper sheath shows that this sheath does not always contain vascular tissue. Among the species that do, we find two forms of vascular skeleton. The bundles entering the upper sheath may be branches from those which pass from cotyledon to hypocotyl, and then they end blindly near the top of the sheath (*Colchicum*). Or the bundles from the cotyledon may themselves enter the sheath, and after a longer or shorter course within it, turn down through the lower sheath to the axis (*Elettaria*, *Commelina*, *Tigridia*). We have described many more examples of the second form than of the first, because the second approaches the imaginary type *X* from which the vascular skeleton of the coleoptile can be derived. In particular, this form of sheath is found in several genera within the Zingiberaceae.<sup>1</sup> We may conclude that this form of vascular skeleton is inherited from some ancestor common to at least some of the genera within the Zingiberaceae. This is the more probable, as the structure of the first node and hypocotyl is also fairly uniform in these genera.

The resemblance between the embryo of *Canna* and that of the Grasses, already pointed out by Hegelmaier ('74, p. 669), is interesting in this connexion, since the Cannaceae are closely related to the Zingiberaceae.

The geographical distribution of the Zingiberaceae indicates that it is an ancient group,<sup>2</sup> and the type of seedling skeleton which is primitive within it probably goes back to an early form of Monocotyledon. This makes the resemblance to the skeleton of Grass seedlings more suggestive, particularly as the likeness extends to first node and hypocotyl.

Schumann points out the remarkable similarity in vegetative characters between the Zingiberaceae and the Gramineae,<sup>3</sup> but he does not therefore assume any genetic connexion. The Scitamineae on the one hand,<sup>4</sup> and the Glumiflorae on the other,<sup>5</sup> are generally considered as natural divisions of the Monocotyledons, without clear affinities to other groups. Both are probably related to the Liliiflorae.<sup>6</sup>

Even if no simple degree of relationship through a common ancestor should be discovered to explain the characters which the Zingiberaceae have in common with the Gramineae, it does not therefore follow that the sheath structure of one group may not illustrate that of the coleoptile in the other. More than one descendant from the prototype of the Liliiflorae may have stiffened its upper sheath by the entrance of whole bundles from the sucker

<sup>1</sup> In the six we have cut, the only exception is *Brachychilum*, in which the sheath contains no vascular tissue at all.

<sup>2</sup> Schumann, K.: Engler's Pflanzenreich, iv. 46; Zingiberaceae, p. 27, 1904.

<sup>3</sup> Schumann, K.: l. c., p. 3.

<sup>4</sup> Petersen, O. G.: Engler's Pflanzenfamilien, ii, Abth. 6, p. 38, 1889.

<sup>5</sup> Hackel, E.: Engler's Pflanzenfamilien, ii, Abth. 2, p. 16, 1887.

<sup>6</sup> Wettstein, R.: Handb. d. syst. Bot., Aufl. 2, p. 782, 1911.

and stalk of the cotyledon, on their way to join the stele of the hypocotyl. The Zingiberaceae may represent one of those forms, the Gramineae another; and the comparatively simple sheath of *Elettaria* may still serve to indicate the manner in which the coleoptile of *Avena* was evolved through a distinct line of descent.

The sheath of *Commelina* may represent an early stage in the evolution of the coleoptile, demonstrating how it might be derived from the simple sheathing base of the cotyledon, in the most usual form of epigeal germination (*Allium*, *Anemarrhena*, &c.). And in this genus, too, interest is at once aroused and baffled by the existence of apparently primitive characters. We have already remarked on the anatomy of the hypocotyl, which is that of a typical Dicotyledon. Solms-Laubach has shown that the stem apex in the embryo of *Commelina* is terminal like that of a Dicotyledon, while in the typical Monocotyledon it is lateral.<sup>1</sup>

In conclusion, we think that the key to the morphology of the Grass embryo lies in the morphology of its seedling, as interpreted by comparison with the seedlings of other Monocotyledons. This comparison is not easy, for the anatomy of the Grass seedling is complicated, and very distinct variants are found within the family. We have shown that all these variants can be derived from an imaginary type *X* (Text-fig. 7, p. 166). The scutellum then represents the sucker of the *X* cotyledon, and the coleoptile its sheath, and in both cases this is the most natural interpretation of their anatomy. The vascular skeleton of *X* is that of a hypogeal Monocotyledon, and is sufficiently near that of the Zingiberaceae to be derived from it without difficulty. But to derive the vascular skeleton of the *Avena* type from that of *X* requires one considerable assumption; that the stalk which should connect scutellum with coleoptile has become united with the hypocotyl.

We maintain that the mesocotyl is more likely to have arisen in this way than as the elongated node, which Van Tieghem suggested ('72). The latter hypothesis does not explain the presence of the inverted trace found within the mesocotyl of certain forms by previous observers.<sup>2</sup> On our view this trace represents the stalk, and is the last vestige of its independence.

<sup>1</sup> Wettstein (l. c., p. 811) remarks on the affinities of the Enantioblastae, including the Commelinaceae, with the Liliiflorae on the one hand and the Gramineae on the other.

<sup>2</sup> Miss Lewin ('87, p. 22 and Pl. III, Fig. 46); Bruns ('92, p. 23); Schlickum ('96, p. 58 and Pl. V, Figs. 147, 157).



LIST IN CHRONOLOGICAL ORDER OF THE PRINCIPAL PAPERS RELATING TO THE EMBRYO AND SEEDLING OF THE GRAMINEAE WHICH HAVE APPEARED SINCE 1872.

- VAN TIEGHEM, PH. ('72): Observations anatomiques sur le cotylédon des Graminées. Ann. des sci. nat., sér. v, Bot., t. xv, pp. 236-76, 2 pl., 1872.
- HEGELMAIER, F. ('74): Zur Entwicklungsgeschichte monokotyledoner Keime, nebst Bemerkungen über die Bildung der Samendeckel. III. *Triticum vulgare*. Bot. Zeit., xxxii, pp. 657-68, 1 pl., 1874.
- WARMING, E. ('79-'80): Forgreningen og Bladstillingen hos Slægten *Nelumbo*. Videnskab. Meddell. fra den naturh. Foren. Kjöbenhavn, 1879-80, pp. 444-55, 1 pl., 1 text-fig. (The author's views regarding the Grass embryo are explained in a footnote to pp. 446-8.)
- KLEBS, G. ('85): Beiträge zur Morphologie und Biologie der Keimung. Untersuchungen aus dem Botanischen Institut zu Tübingen, Bd. i, Heft 4, pp. 536-635, 24 text-figs., 1885.
- LEWIN, M. ('87): Bidrag till Hjertbladets Anatomi hos Monokotyledonerna. Bihang till K. Svenska Vet.-Akad. Handlingar, Bd. xii, Afd. iii, No. 3, 28 pp., 3 pl., 1887 (for 1886).
- HACKEL, E. ('87): Gramineae, in Die natürl. Pflanzenfamilien, von A. Engler und K. Prantl. Teil ii, Abth. ii, pp. 10-14, 1887.
- TSCHIRCH, A. ('90): Die Saugorgane der Scitamineen-Samen. Sitzungsber. d. k. preuss. Akad. d. Wissenschaften zu Berlin, Jahrg. 1890, Erster Halbband, pp. 131-40, 1890.
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- CELAKOVSKÝ, L. J. ('97): Ueber die Homologien des Grasembryos. Bot. Zeit., lv, Abth. I pp. 141-74, 1 pl., 1897.
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- SARGANT, E., and ROBERTSON, A. ('05): The Anatomy of the Scutellum in *Zea mays*. Ann. of Bot., vol. xix, pp. 115-123, 1 pl., 1905.
- KIRCHNER, O. VON, LOEW, E., and SCHRÖTER, C. ('08-'12): Lebensgeschichte der Blütenpflanzen Mitteleuropas. Bd. i, Abt. ii, Bogen 1-18. Gramineae, pp. 1-288, 399 text-figs., 1908, 1909, 1912.

EXPLANATION OF FIGURES IN PLATES IX, X.

Illustrating the paper by Miss Sargent and Mrs. Arber on the Comparative Morphology of the Embryo and Seedling in the Gramineae.

The lettering throughout the Plates is uniform: *sc.*, main bundle of scutellum; *sc'*, trace of scutellum in axis; *P, P'*, coleoptile bundles; *M*, midrib of first leaf; *L<sub>1</sub>, L<sub>2</sub>, L<sub>3</sub>*, lateral traces from first leaf on one side of *M*; *L'<sub>1</sub>, L'<sub>2</sub>, L'<sub>3</sub>*, lateral traces from first leaf on other side of *M*; *r., r.*, cauline roots; *rx.*, root-xylem; *rp., rp.*, root-plates.

PLATE IX.

*Avena sativa*, L.

Fig. 1. Coleoptile bundle from transverse section through young seedling, in which plumule is entirely enclosed within coleoptile. The phloem groups are deeply stained because they are full of proteids, and they are quite distinct (*ph.*, *ph.*). One group of protoxylem (*px.*). × 250.

Fig. 2. Transverse section of coleoptile bundle from older seedling. Two groups of metaxylem (*met.*, *met.*), and scattered *px.* elements between them.  $\times 250$ .

Fig. 3. Transverse section of first node from same seedling as Fig. 2. Xylem of *P* and *P'* is branching: the outward branches will form xylem of *sc.*'; the inward branches that of the coleoptile trace within the stele (*x.*).  $\times 155$ .

Fig. 4. Mesocotyl of same seedling a little below the first node. Traces *M* and *x* face each other in the stele; they are bordered by a well-defined root-plate on either side. The inverted scutellum trace (*sc.*') is quite distinct from the stele.  $\times 64$ .

Fig. 5. Stele and scutellum trace more highly magnified a little below Fig. 4 in the same seedling. Scutellum trace with two distinct phloem groups, two groups of metaxylem, one of protoxylem. Root-plates very clear (*rp.*, *rp.*) in stele.  $\times 150$ .

*Zizania aquatica*, L.

Fig. 6. Drawing of seedling, life-size. *A.*, grain; *R.*, primary root; *Ep.*, epiblast; *Pl.*, plumule; *col.*, coleoptile. The dotted line *A.A.* indicates level of Figs. 7 and 8.

Fig. 7. Diagram of first node from seedling drawn in Fig. 6. The section is taken at level *A.A.* (Fig. 6). Lettering as in *Avena*.  $\times 40$ .

Fig. 8. Detail of the space enclosed by dotted lines in Fig. 7. Letters as before.  $\times 240$ .

PLATE X.

*Zizania aquatica*, L.

Fig. 9. Vascular girdle from older seedling. The coleoptile traces *P* and *P'* have not yet entered the stele. They are approaching it in direction indicated by arrows. Cauline root given off at *r.*  $\times 66$ .

*Sorghum vulgare*, Pers.

Fig. 10. Mesocotyl of young seedling drawn in Text-fig. 20, p. 186. Transverse section a little above insertion of scutellum.  $\times 260$ . *px*<sub>1</sub>, protoxylem of scutellum trace within stele; *px*<sub>2</sub>, protoxylem of downward coleoptile traces; *px*<sub>3</sub>, protoxylem of *M*, which is midrib of first leaf; *rx.*, *rx.*, 'sentinel vessels' of root-xylem.

Fig. 11. Coleoptile bundle from older seedling. Two phloem groups very distinct.  $\times 320$ .

Fig. 12. First node, from same seedling as Fig. 11. The xylem of the coleoptile bundles *P* and *P'* forms a bridge across the stele. Some thin-walled elements of large lumen appear at periphery of stele (root-xylem).  $\times 75$ .

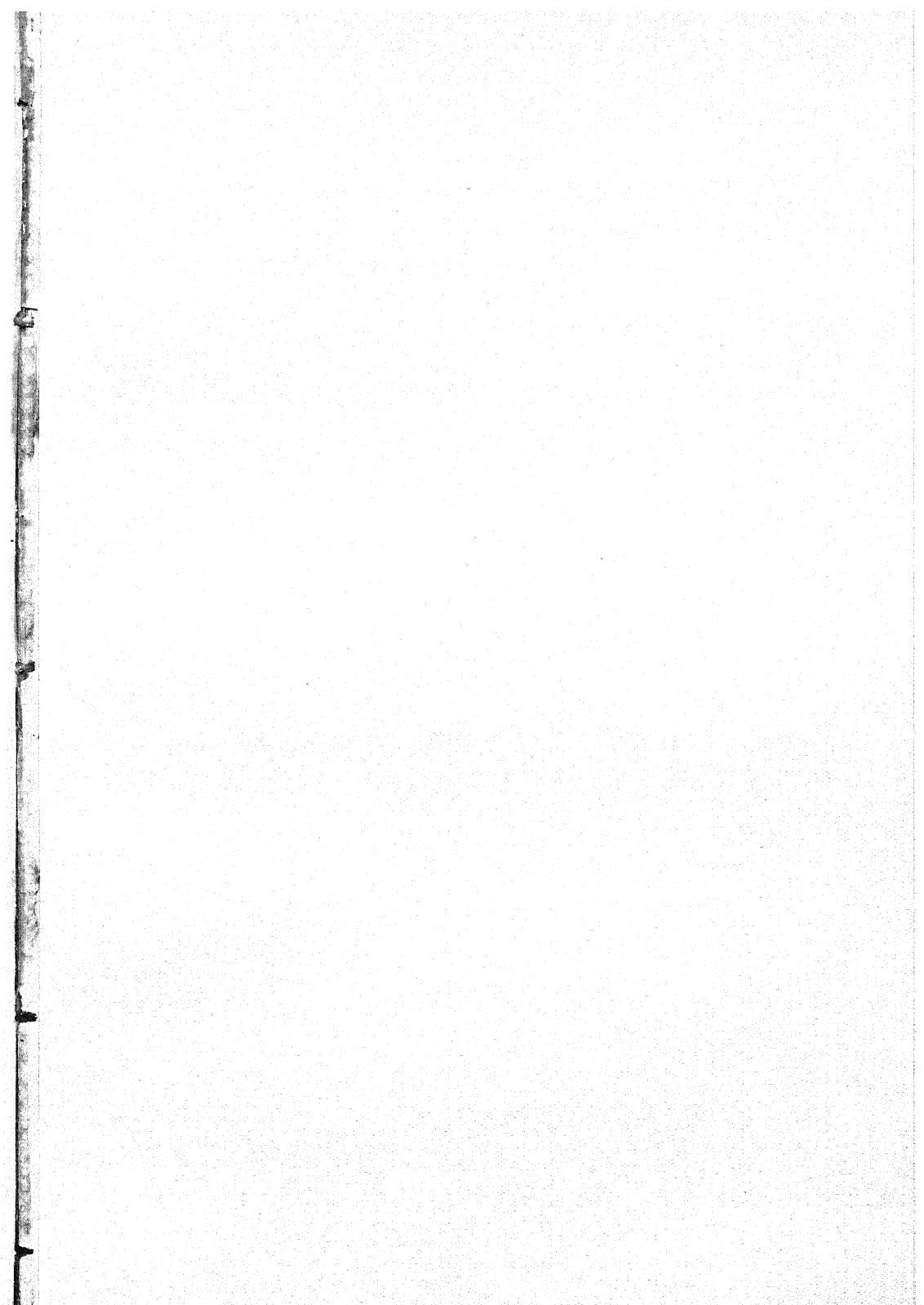
*Anomum angustifolium*, Sonner.

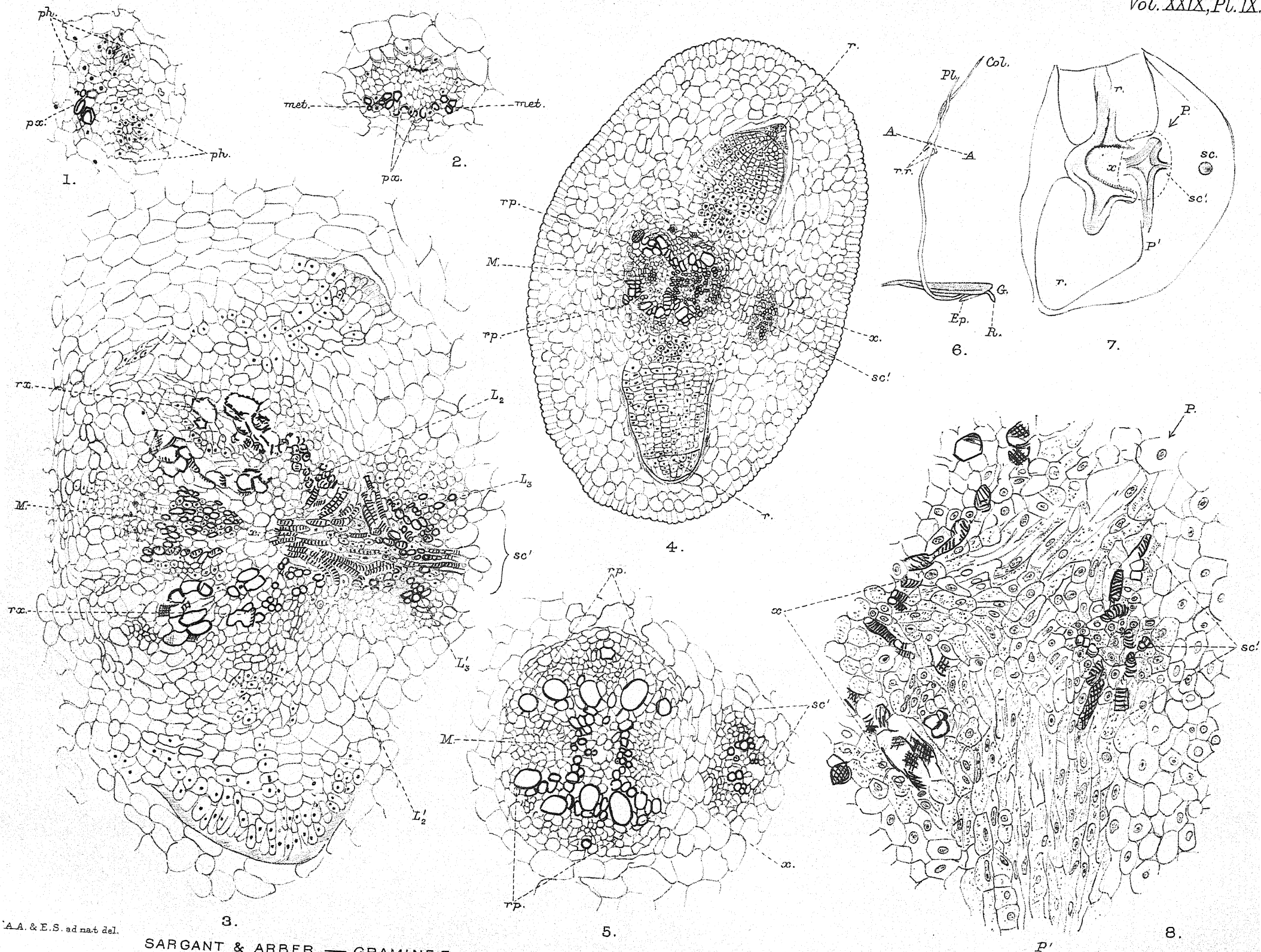
Fig. 13. Transverse section just above first node. *P* and *P'* are branching to meet cortical traces. The xylem girdle is nearly complete on one side; on the other there is an isolated cortical bundle (*c.*), which links up with the girdle a few sections lower in the series.  $\times 190$ .

Fig. 14. Hypocotylar stele of same seedling from same series, not far below first node. Large group of protoxylem, common to all the traces, in centre. The arrows (*m.r.*) point to pathways of clear tissue dividing the traces, and partially bordered on either side by metaxylem.  $\times 190$ .

*Tigridia Pringlei*, S. Wats.

Fig. 15. Transverse section through bundle of cotyledonary sheath in upper part of its course. It has doubled on itself, and its protoxylem is cut twice (*px.*, *px.*), once going upwards, and a second time on its return. Lower down, the two segments separate. (Compare Text-fig. 8, p. 168.)  $\times 260$ .

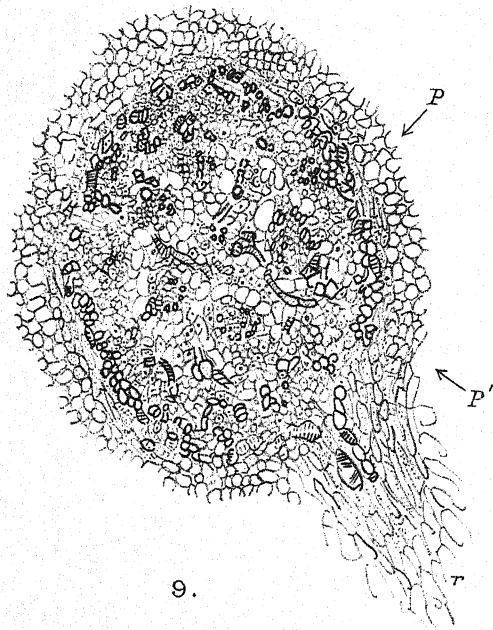




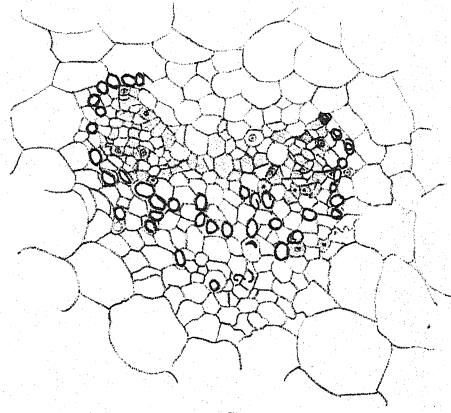
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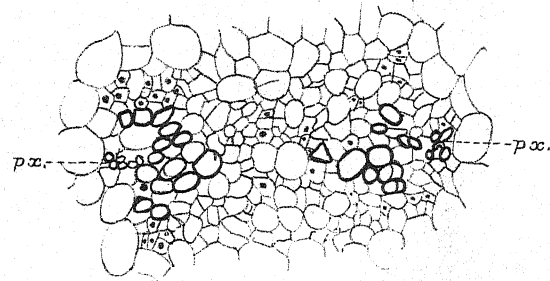
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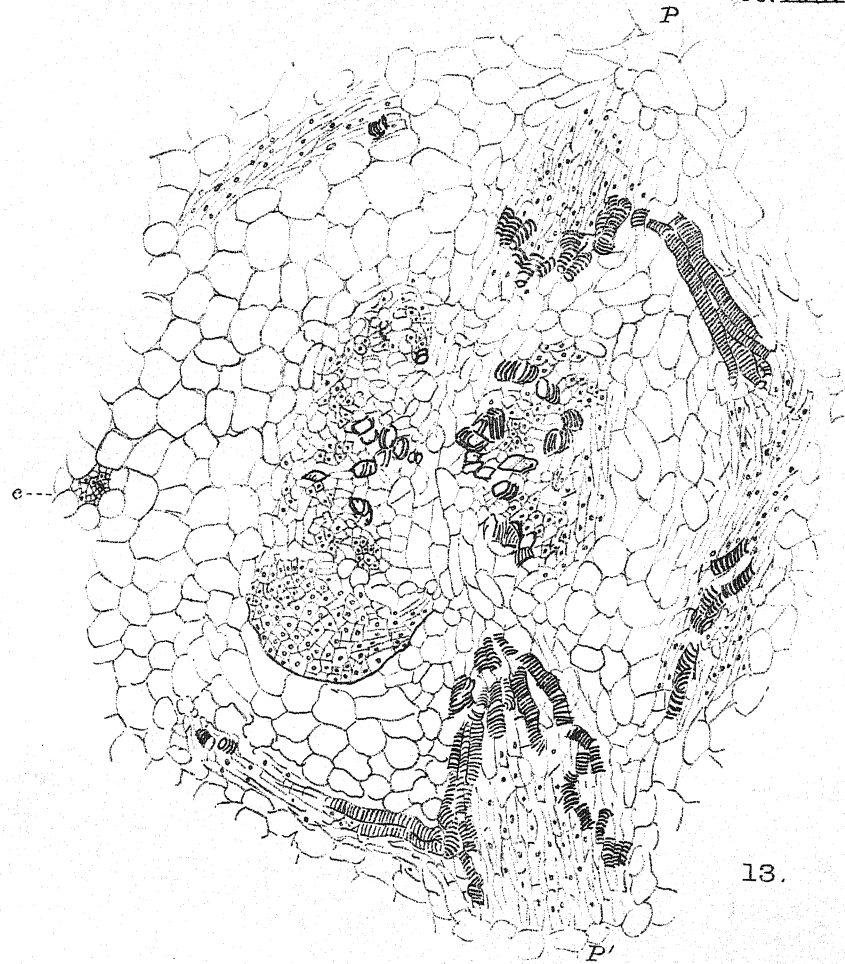
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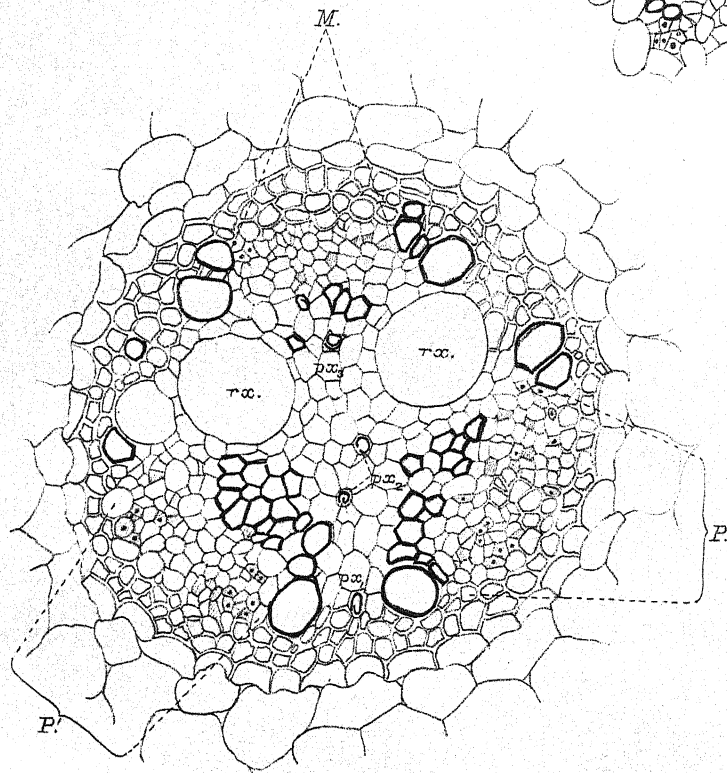
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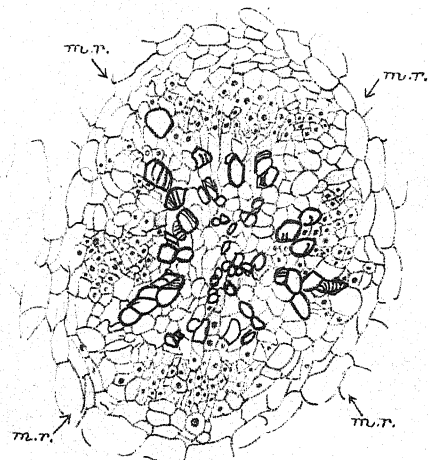
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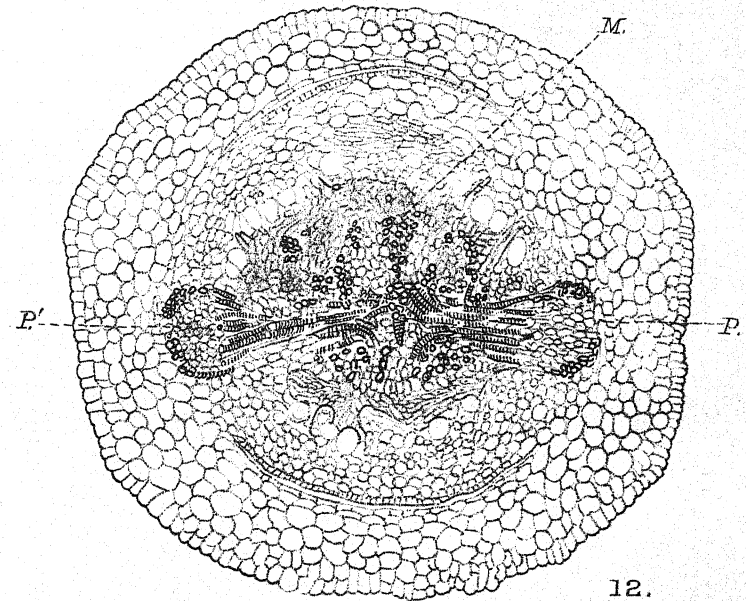
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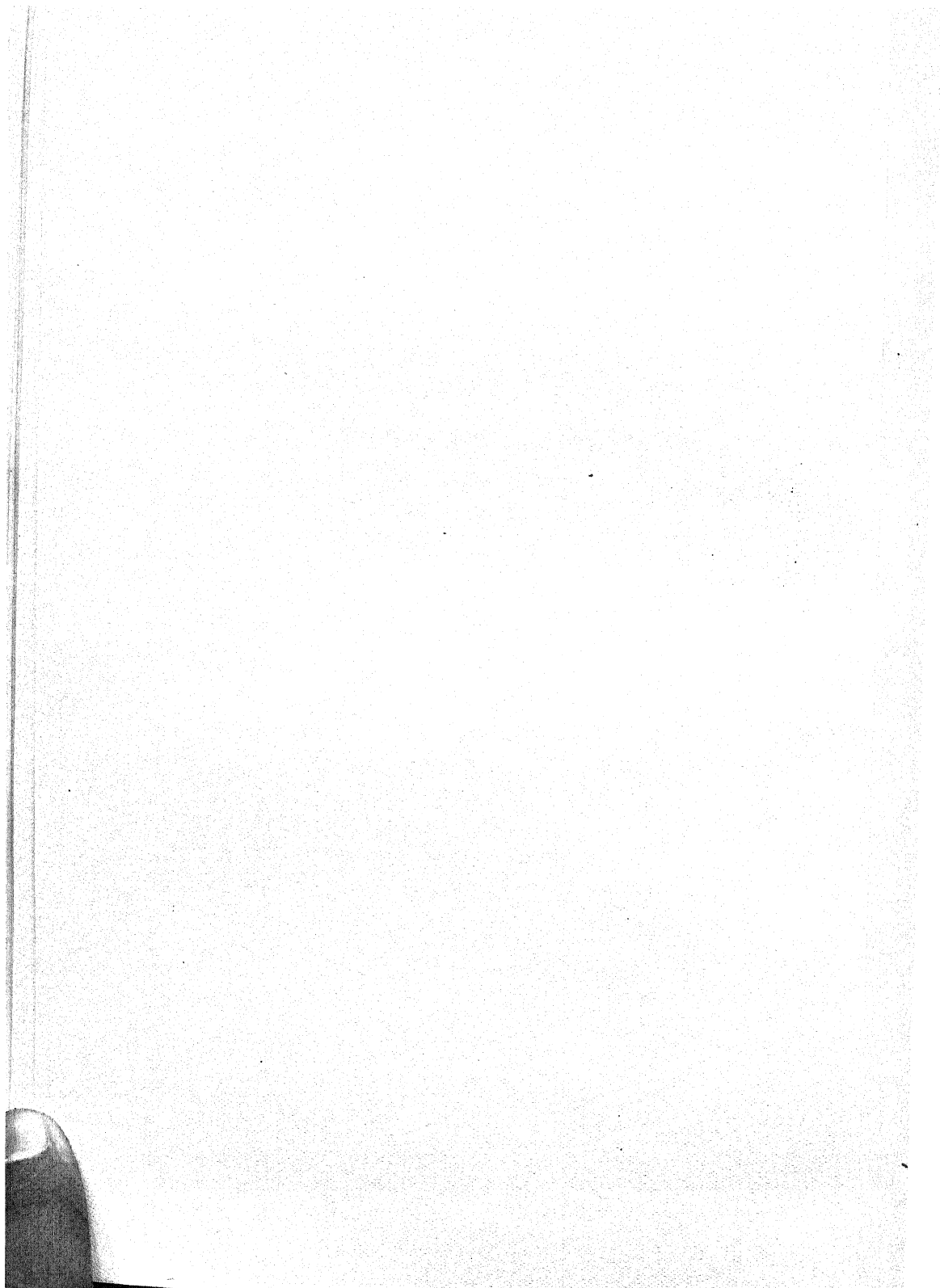


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# The Branching and Branch Shedding of *Bothrodendron*.

BY

MARJORIE LINDSEY.

With Plate XI and three Figures in the Text.

## INTRODUCTION.

THE object of this paper is to bring forward some new evidence with regard to the ulodendroid scars of *Bothrodendron*, and to discuss the ulodendroid condition both in this genus and in *Lepidodendron*.

From very early in the history of Palaeobotany these two genera have been known to bear, in certain cases, two opposite rows of depressed circular scars on their main stems.

Such stems were said to be in the ulodendroid condition, and practically every possible type of appendicular organ has, at one time or another, been suggested as the cause of the scars.

The various theories regarding the ulodendroid scars may therefore be grouped under five heads, representing the five possible types of appendicular organ.

We have then :—

1. *The floral theory*, put forward by Rhode, and agreed to by Allan (1), who compared the plant with a cactus.
2. *The root theory*, first suggested by Brongniart and more fully discussed by Carruthers (3). This was refuted by Williamson (14) and Thompson (11).
3. *The bulbil theory* of Stur (10), which was in its turn refuted by Schimper (8).
4. *The cone theory* due in the first place to Lindley and Hutton (5), but confirmed by many others, notably Thompson (11). For long this was the accepted theory, but it has recently been shown to be improbable by Watson (12) and Renier (7), who prefer
5. *The branch theory*, originally due to Sternberg.



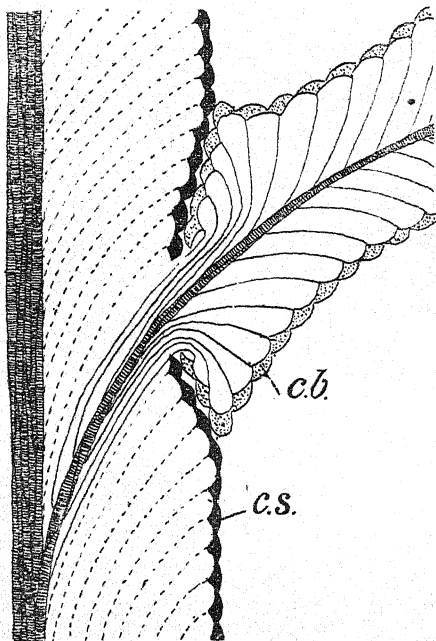
## THE BRANCH THEORIES OF THE ORIGIN OF THE SCAR.

In 1907 D. M. S. Watson (12) described a specimen of *Bothrodendron* in the Manchester Museum, and explained that it showed the ulodendroid scar to be left by a dehiscient branch, the base of which occupied the whole area of the scar.

The surface of the scar showed leaf-traces running out to the branch.

M. Renier (7) in his monograph described a specimen in which a branch of *Bothrodendron* was seen on one side 'of a very thin plate of shale', while

in the same position, on the other side, was a ulodendroid scar with an eccentric umbilicus. He describes this as indisputable evidence in favour of the branch theory. But, arguing from specimens of *Lepidodendron*, his conclusions as to the way in which the branch was joined to the stem show that his conception of a branch theory differs fundamentally from Watson's. The one (Watson's) supposes the branch to have been attached to the whole area of the scar, and to have been provided with some branch-shedding mechanism such as a layer of cork, and may therefore be described as the *Abscission Layer Theory*.



TEXT-FIG. 1. Illustrates the umbilical attachment theory. *c.b.*, cortex of branch; *c.s.*, cortex of stem; leaf-traces of stem are represented by dotted lines, leaf-traces of branch by continuous lines; the principal vascular axes are shaded.

The other (Renier's) supposes the branch to have been attached to the stem by the umbilicus alone, that the branch 'had a conical base like the branch of

a calamite', and that the rest of the scar was formed by pressure as the branch and trunk grew in size simultaneously. This may therefore be known as the *Umbilical Attachment Theory*.

Text-figs. 1 and 2 show the essential differences between these two theories; in the case of the umbilical attachment theory the cortex of the branch was supposed to adhere to that of the stem over the area of the scar. Therefore the markings on the scar represent the markings on the inner side of the outer cortex of the branch, and bear no relationship with the leaf-traces of the stem.



In a more recent paper in the *Annals of Botany*, July, 1914, Mr. Watson (13) has discussed these two theories, objecting to the umbilical attachment theory on the following grounds:

1. The relative insignificance of the secondary thickening in any lepidodendroid plant as compared with that which would be required by M. Renier's theory.

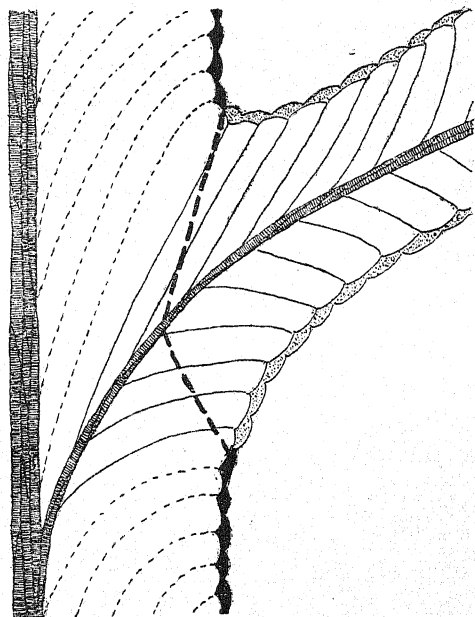
2. No ulodendroid scars are known in which the diameter of the umbilicus is more than a quarter of the diameter of the scar; that is, the first stages as required by the umbilical attachment theory are unknown.

3. The weakness of the calamite branch analogy.

4. The evidence of structure material shows no contraction at the base.

5. In the two new sections described by Mr. Watson the whole base of branch is cut off by a thick layer of secondary tissue; that is, there is a definite abscission layer.

There are only two additional arguments which I should like to bring forward. For the sake of argument, Mr. Watson admits M. Renier's contention that the leaf-trace markings on both halves of his specimen do not correspond in position. But if the figure of



TEXT-FIG. 2. Illustrates the abscission layer theory. The heavy broken line marks position of abscission layer; otherwise as in Text-fig. 1.

one half of the specimen is traced and the tracing reversed on to the figure of the other half, it will be seen that the leaf-trace markings agree very closely in position and are equal in number.

The second point concerns the arrangement of the leaf-trace markings on the scar. In the specimens figured by M. Renier and in many other well-known figures such as those of Stur, the quincuncial arrangement of the leaf-traces on the trunk is continued on the lower part of the scar, and this was used as an argument that the scar, except the umbilicus, was of the same nature as the trunk—that it was, in fact, merely a flattened portion thereof. But this does not take into consideration the fact that on the upper part of the scar the leaf-traces are very differently arranged. On the abscission layer theory the whole area of the scar simply represents

the plane of separation of a fallen branch, and the leaf-traces passing out to the branch would of necessity cut this plane of separation and leave their impressions thereon. The leaf-trace markings on the lower part of the scar would be more or less in continuity with those on the stem below, because the leaf-traces belong to the same phyllotactic series in both and cut the abscission layer and the stem at approximately the same angle in both. On the upper part of the scar, however, it will be seen from Text-fig. 2 that the leaf-traces run almost parallel to the plane of the scar, and so they would appear not as small punctations or dots, but as an irregular series of elongated scars.

#### DESCRIPTION OF TWO NEW SPECIMENS.

There are in the Manchester Museum two hitherto undescribed specimens of *Bothrodendron minutifolium*; these two new specimens are among the finest known. The first consists of a large branch some fifteen inches in length (Pl. XI, Fig. 1). This was partly exposed in a matrix of shale, which, being very easily split, allowed further portions of the branch to be exposed on development with a small chisel.

The main stem is about two inches in diameter, and at a distance of some six inches from the lower end of the specimen it branches dichotomously (Pl. XI, Fig. 2). The left branch dichotomizes again almost immediately, giving the appearance of three equal branches. Slightly further up, these three all dichotomize freely, forming a bushy mass of small branches, so that further development in this region merely leads to further branches being disclosed below on successive layers of shale.

The upper portions of the branch show the typical *Bothrodendron minutifolium* foliage (Pl. XI, Fig. 3). Lower down, this foliage has fallen off, leaving the spirally arranged oval scars plainly visible. These scars have the three punctations representing the vascular bundle and the parichnos, and above each is seen the impression of the ligular pit.

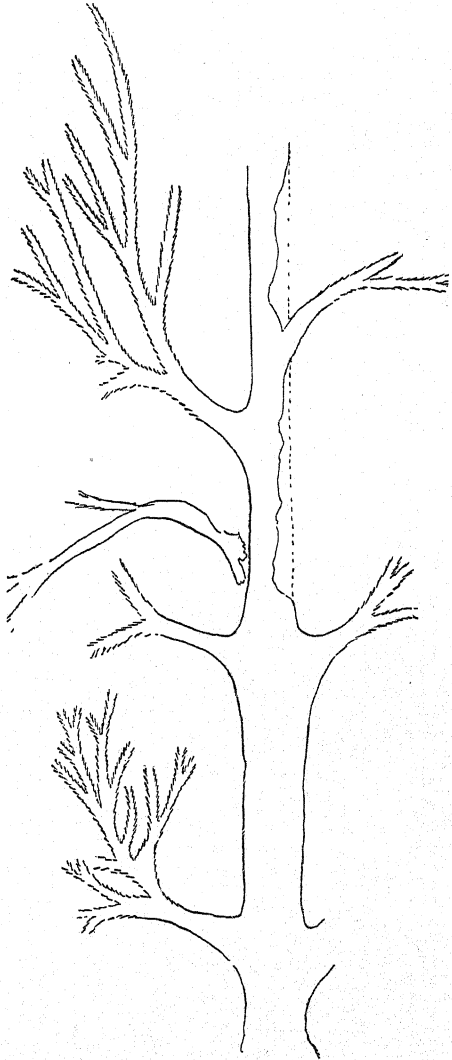
They are separated from each other by about half an inch, and the area between is marked by transverse furrows. As was mentioned by Zeiller (15), when more highly magnified the ridges between the furrows are seen to be covered by a number of raised circular structures, with a depression in the centre of each. It is not quite clear what these represent, but they are possibly in the nature of stomata.

But it is the base of the specimen which renders it so valuable (Pl. XI, Fig. 2). The base of the stem on development was found gradually to broaden out into a trumpet-shaped body, and then to end quite suddenly and cleanly in a convex edge, which corresponds in size with the diameter of an ordinary ulodendroid scar. This convex edge was not due to accident in fossilization, but was really the true ending of the branch. The rest of

the preservation is so excellent that had the branch been any longer or in any way different in form, it would most certainly have shown the fact quite clearly.

The other specimen (Pl. XI, Fig. 4) is of rather a different nature: it consists of what appears to be the termination of a main trunk, which gives off branches in two opposite and alternate rows—an entirely new form of branching for a *Bothrodendron*.

The various surface features, such as leaf scars, are nearly as well preserved as in the first specimen. The main axis is about fourteen inches in length and is an inch in diameter in its widest part. There are five branches well shown, and these each have a broadened trumpet-shaped base. Some are seen to dichotomize at a short distance from the main stem. (See Text-fig. 3.) Others show clear evidence of the spreading, bushy mass of small branches which characterized the other specimen. This is well shown in the lowest branch exposed. Here we have a branch the base of which gradually broadens out until it is about two and a half times the size of the branch itself in diameter. This is exactly the relation in size of the branch to its base in the first specimen, and at a similar distance in both the branches dichotomize and spread out. In both, also, further branches below are exposed on excavation, and in both the foliage is retained on the upper portion, while lower down it has fallen away, leaving typical leaf scars.



TEXT-FIG. 3. *Bothrodendron minutifolium*.  
 $\frac{1}{3}$  nat. size.

A very important point to notice in connexion with this specimen is the fact that the cortex of the branch is continuous with that of the main

stem. From this it seems clear that the branch was attached to the stem in a quite normal way, and not in the manner in which M. Renier supposes, for in the latter case there would be a distinct ring where the cortex of the stem joined that of the branch.

It seems reasonably certain that while the second specimen is a main stem with the branches attached, the first is a branch which has fallen off. Such a branch on falling would leave a ulodendroid scar. Supposing all the branches were to fall off the second specimen you would then have two alternate rows of scars on opposite sides of a main axis just as is most usual in ulodendroid stems.

In regard to this, however, one question crops up, and that is in connexion with the spacing of such scars. Should the branches fall from the second specimen, the distance between consecutive scars would be considerable, and though not as large as appears at first sight owing to the bases of the branches spreading out as they do, still it would leave larger spaces between than occur in the stems figured by M. Renier. In these the scars are practically contiguous, as they are in a good many specimens figured. But this close arrangement is not universal. In the Manchester Museum, it is true, there are a certain number of examples of this type, but there are also others in which the scars have a separation of eight or nine inches. There is unfortunately no series of scars of the size such as would be left on specimen 2 (which is obviously far from full-grown); the most usual is that of specimen 1, i. e. about  $3\frac{3}{4}$  inches in diameter. Taking, then, six specimens which showed scars of approximately this size, the distances between consecutive scars were measured with the following results:

| A. Diameter<br>of Scar. | B. Distance from<br>bottom of scar to<br>the top of the scar<br>below. | C. Index<br>$= \frac{A}{B}$ . |
|-------------------------|--|-------------------------------|
| 1. 3·8"                 | 9·5"   | 0·30                          |
| 2. 3·6"                 | 8·1"   | 0·33                          |
| 3. 4·1"                 | 7·0"   | 0·58                          |
| 4. 3·5"                 | 5·4"   | 0·66                          |
| 5. 3·8"                 | 2·9"   | 1·31                          |
| 6. 3·9"                 | 2·3"   | 1·69                          |

It would therefore seem as if the distance between one scar and the next was not constant, but probably depended on conditions of growth, or possibly on the genus of the specimen, for in the specimens in the Manchester Museum it is noticeable that the scars on *Bothrodendron* are, on the whole, further apart than those of *Ulodendron* proper, and it has already been pointed out that the specimens figured by Renier are *Lepidodendron*, whereas the two new specimens are *Bothrodendron*. There are not, however, enough specimens of *Ulodendron majus* (i. e. the lepidodendroid form) from which to state this as a definite assertion, but it does seem quite reasonable.

RELATION OF *BOTHRODENDRON PUNCTATUM* TO *BOTHRODENDRON MINUTIFOLIUM*.

In the paper by Renier (7) mentioned before, he states that *Bothrodendron punctatum* and *Bothrodendron minutifolium* are in reality only one species—in fact, that he has found both types of surface-marking on the same specimen. In both, the leaf scars are practically the same, the difference between them being (a) the bark (which in *minutifolium* shows transverse furrows, while *punctatum* is said to be longitudinally marked), and (b) the fact that *Bothrodendron punctatum* has ulodendroid scars, while *Bothrodendron minutifolium* has none.

Decidedly *B. minutifolium* shows transverse markings on the bark; but whether the markings in *B. punctatum* are of the same nature or due to a splitting of the bark consequent on growth, as was suggested by Renier, I am unable to say from observations, but it does seem quite reasonable.

As to the other point, (b), in many cases of ulodendroid *Bothrodendrons* the surface is not sufficiently well preserved for these furrows to be observed, and so it may be that many large stems bearing scars may have had either one kind of marking or the other, the presence of scars being the sole reason for their being called *punctatum*.

In any case the two species are obviously closely allied, and it is quite probable that what happens in one species in such an important matter as branch-shedding will have its counterpart in the other.

Therefore if *Bothrodendron punctatum* had ulodendroid scars it is at least probable that *Bothrodendron minutifolium* had also; hence the fact that the two new specimens here described are *Bothrodendron minutifolium* need be no serious argument against their being evidence in favour of the abscission layer theory of the ulodendroid scar.

## SUMMARY.

In the foregoing paper two new specimens of *Bothrodendron minutifolium* are described—one showing branching of a type hitherto undescribed. It consists of the end of a main axis with opposite rows of alternate branches with trumpet-shaped bases. The cortex of the main stem is continuous with that of the branches, showing the branches to be attached in quite a normal way. These branches themselves show the ordinary bushy, spreading mass of small branches usual in known *Bothrodendrons*.

It is equally clear that the other specimen is a similar though larger branch which has fallen away—its clean-cut, trumpet-shaped ending suggesting that it has broken away along a definite abscission layer.

Though previously described *Bothrodendrons* in the ulodendroid condition have been attributed to *Bothrodendron punctatum*, the fact that

these new specimens are *Bothrodendron minutifolium* is not an insurmountable difficulty, since these two species, if not identical, are at any rate very closely allied, and it is therefore quite probable that both had the same method of shedding.

Finally, I wish to express my thanks to Dr. Hickling for his assistance in the preparation of this paper, and also for the photographs with which it is illustrated.

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THE UNIVERSITY, MANCHESTER.

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# DESCRIPTION OF PLATE XI.

Illustrating Miss Marjorie Lindsey's paper on the Branching and Branch Shedding of *Bothrodendron*.

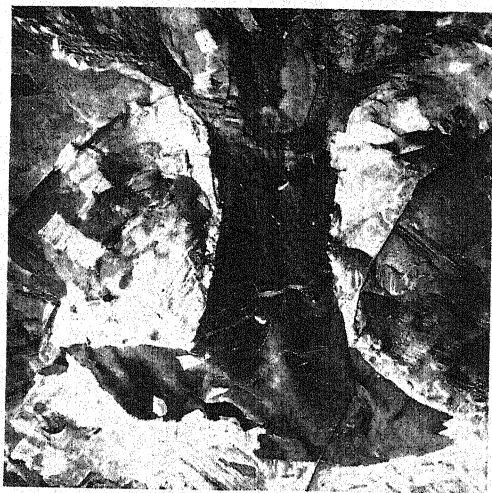
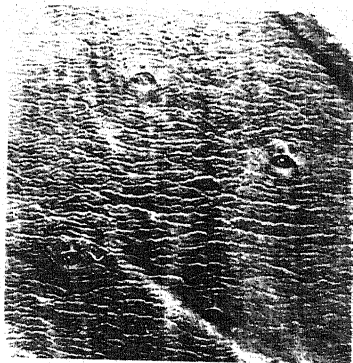
- Fig. 1. First specimen of *Bothrodendron minutifolium*, showing the bushy nature of the branch.
- Fig. 2. The base of the first specimen, showing the dichotomy and the trumpet-shaped ending.
- Fig. 3. Part of the surface of *Bothrodendron minutifolium*, showing leaf cushions with vascular scars and the ligular pit, and also the furrows in between the scars.
- Fig. 4. The second specimen of *Bothrodendron minutifolium*, showing the main axis with alternate branches on each side, each branch of a spreading, bushy nature and with a trumpet-shaped base.



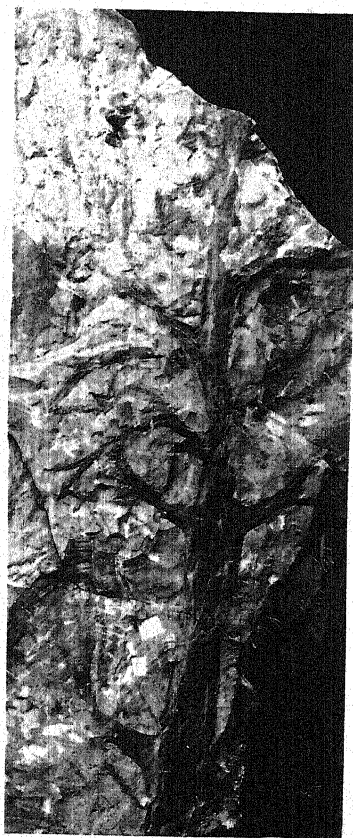
1.



3.



2.



4.

Huth coll.

LINDSEY-BOTHRODENDRON.





## A Second Contribution to our Knowledge of the Anatomy of the Cone and Fertile Stem of *Equisetum*.

BY

ISABEL M. P. BROWNE.

With Plates XII-XIV and five Figures in the Text.

I should like to prefix to this Second Contribution an expression of regret that, owing to a printer's error, Text-fig. 2 of my earlier paper on *Equisetum* was printed upside down (Browne, p. 669).

### MATERIAL.

THE present investigations were confined to *Equisetum maximum*, Lam. (*E. Telmateia*, Ehr.). Two complete cones were cut serially into transverse sections; these I shall henceforward term Cones A and B. Cone A was young; about one-half of it was covered by the uppermost whorl of leaves of the fertile stem. Including the terminal group of undifferentiated or congenitally concrescent sporangiophores, the length of the cone was about 1.5 inches; the actual height of the axial stele, a longitudinal reconstruction of the xylem of which was made, was 1.15 inches. At its widest the stele of the axis of this cone had a diameter of about 4.75 mm. At the lower end the destruction of the pith—owing to the growth in diameter of the stem—had only just been initiated. The degree of disturbance of the central cells of the pith varied a little locally, but relatively few cells were involved, and at this early stage the pith cannot be said to be definitely fistular in any part of the cone. As we pass upwards the cells of the pith gradually cease to be destroyed or to suffer distortion. Cone B was a very large one and fully mature, though the apical 'drip-point' remained, as it seems always to remain, externally undifferentiated into distinct sporangiophores. The longitudinal reconstruction of the xylem extended from the supra-annular fusions (Browne, p. 690-4) to the point at which a single, large, terminal strand enters the terminal 'drip-point', a height of just under three inches; including the latter, the height of the cone was over three inches. Though this cone was large, it was by no means exceptional in respect of its size. Of a branching cone, C, the upper part, which included the region of branching, was cut serially. The region

transitional from a large cone, Cone D, to the fertile stem was also cut serially, and proved abnormal in several respects. Serial longitudinal sections were made of a normal cone, E. Besides some hand-sections, serial sections of portions of four normal cones were cut, but as their study only confirmed the conclusions from Cones A and B no description of them seems necessary. Serial sections of a mature node of a sterile stem were also prepared for comparison with the fertile stem below Cone D.

#### ANATOMY OF THE CONE.

The longitudinal reconstructions of the xylem in Cones A and B (cf. Pl. XII and Pl. XIII) show that the whorls of traces are not inserted truly horizontally on the axial stele; this appearance is not due to the obliquity of the sections, as the appearance of the individual tracheides is that of the structures cut horizontally. The insertion, both of the traces and of the sporangiophores, is often distinctly irregular, but much more so in the large Cone B than in Cone A. In the lower region, where the elongation of the internodes is naturally greater than in the upper part, it is often easy to recognize the traces that belong to any one whorl. In Cone A there seem to have been nineteen whorls, excluding the terminal cluster of incompletely differentiated sporangiophores. In Cone B, owing to the much greater irregularity in the disposition of the traces and of the sporangiophores, it is very difficult to estimate the number of whorls present. The impossibility of saying with any certainty to what whorl many of the traces belong makes it difficult to say of what order are the parenchymatous meshes arising above some of them, or, in other words, to say into what number of internodes these meshes extend. Thus, in Cone B, many of the whorls of traces are duplicated over a portion of their extent; when this duplication takes place freely, we have what may be termed a pseudo-whorl; that is to say, while the increase in the number of the traces in such a region, compared with the number of traces in other whorls in that part of the cone and with the diameter of the stele, is too great for us to regard this phenomenon as merely an unimportant variation leading to a slight increase in the number of traces in a whorl, the number of supernumerary traces that have been called into existence is not sufficiently large for them to constitute an independent whorl strictly equivalent to other whorls in that part of the cone. I estimate that there are about twenty-eight whorls in Cone B, three of which are pseudo-whorls. Were lines delimiting these whorls included in Pl. XIII they would be very sinuous and irregular; as, however, their course would largely depend on individual interpretation, they have not been marked. A less great increase in the number of traces of a whorl, though an increase leading to the development of traces, situated, as are those of a pseudo-whorl, a little above other traces of the whorl, I have considered merely as a local dupli-

cation of that whorl. What I want to emphasize is that the difference between a pseudo-whorl and a local duplication of a whorl is one of degree; consequently, in the classification of a group of traces, the drawing of a dividing line between a pseudo-whorl and a duplicated whorl is a matter of individual interpretation, and the classification is inevitably in some cases an arbitrary one. It is obvious that by regarding a group of traces as a duplication of an existing whorl or as an independent whorl (pseudo-whorl), a given mesh may be regarded as of the order  $x$ , or of the order  $x+1$ . Even when this factor does not come into play, parenchymatous meshes of the same actual length may, in the same region of the cone, belong to different orders, and this for three reasons. Firstly, because the sporangiophores and traces are inserted so irregularly on the axis and axial stele that different portions of the same internode may vary considerably in height. Secondly, because parenchymatous meshes may arise and be closed at very different distances above the departure of a trace; occasionally a mesh makes its appearance at such a height above a trace that it originates but little below the level of departure of the traces of the next whorl above, and, if it is closed half-way up this second internode, this mesh of the second order is actually shorter than a mesh of the first order, extending throughout nearly the whole of one internode. Lastly, because the tendency of meshes to become decurrent downwards and to one side of traces above which they may be considered to have originated in the phylogeny—a tendency noted in the study of the cones of *E. palustre* and *E. limosum* (Browne, p. 673) is a well-marked character of cones of *E. maximum*—and often increases the length of a mesh without bringing it under the category of a mesh of a higher order. These difficulties in the estimation of the height of the meshes, particularly in the classification of those of Cone B, must be borne in mind in studying the following analysis of the nature and number of these meshes.

MESHES ORIGINATING WITHIN THE CONES THEMSELVES.

(i. e. above the lowest whorl of sporangiophores.)

| Orders : | 1  | 2  | 3  | 4  | 5  | 6 | 7 | 8 | 9 | 10 | 11 | 17 | Total. |
|----------|----|----|----|----|----|---|---|---|---|----|----|----|--------|
| Cone A   | 29 | 30 | 12 | 7  | 5  | 2 | 3 | 0 | 0 | 0  | 0  | 0  | 88     |
| Cone B   | 33 | 46 | 32 | 23 | 10 | 5 | 5 | 4 | 3 | 2  | 3  | 1  | 167    |

This table does not give the full number of meshes extending into the cone, for some of the meshes arising above the annulus, and some of those arising above the uppermost whorl of leaves, persist into the cone for varying distances. The number and nature of these two sets of meshes arising respectively above the annulus and above the uppermost whorl of leaves are given in the two following tables. Those, in the first of these two tables, of orders higher than the first order, and in the second table of orders higher than

the second order, persist into the cone itself, i. e. into the internode above the lowest sporangiophores.

#### MESHES ORIGINATING ABOVE THE ANNULUS.

(The space between the annulus and the lowest fertile whorl is considered as an internode.)

| Orders : | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Total. |
|----------|---|---|---|---|---|---|---|---|--------|
| Cone A   | 1 | 3 | 2 | 1 | 0 | 0 | 0 | 0 | 7      |
| Cone B   | 0 | 1 | 3 | 1 | 2 | 1 | 1 | 1 | 10     |

#### MESHES ORIGINATING ABOVE THE LAST LEAFY WHORL.

(The spaces between the last leaf-whorl and the annulus, and between the latter and the lowest fertile whorl, are considered as internodes.)

| Orders : | 1  | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 17 | Total. |
|----------|----|---|---|---|---|---|---|---|---|----|----|----|--------|
| Cone A   | 6  | 5 | 4 | 2 | 5 | 0 | 1 | 0 | 1 | 0  | 0  | 0  | 24     |
| Cone B   | 10 | 9 | 7 | 5 | 3 | 2 | 1 | 1 | 0 | 1  | 1  | 1  | 41     |

#### GENERAL CHARACTERS OF THE XYLEM OF THE CONE.

The individual tracheides of the cone of *E. maximum* resemble in structure those of the other cones of *Equisetum* studied by me, viz. *E. arvense*, *E. palustre*, and *E. limosum*; that is to say, they are provided with spiral or annular thickenings. In the species at present under consideration they are markedly less strongly lignified, and their walls are frequently only slightly thickened. In mature cones their average diameter is slightly less than in the three species already studied. Moreover, there are more parenchymatous cells mingled with them than with the tracheides of the other species. In comparing the reconstructions of the xylem of the cones of *E. maximum* with the reconstructions of the xylem of such a species as *E. arvense*, we should bear in mind that in the latter species the xylem constitutes a more or less solid cylinder, though one of little radial depth, while in the former the xylem forms a cylinder consisting chiefly of woody cells, but also of numerous patches of unlignified parenchyma. These patches are too small to be shown in the reconstructions of the xylem; they are commonest at the extreme base of large cones such as Cones B and D (see Pl. XIV, Fig. 3). In places, it is true, and oftenest near the point of departure of a trace, the xylem of a bundle forms a more or less continuous band (see Pl. XIV, Fig. 1). Detached groups of tracheides, usually of small calibre, occur more internally in the bundle, as they do in all the species studied, but rather more frequently than in these. Such groups of small tracheides tend to become torn and disorganized very early, before the formation of definite carinal canals. The traces of the sporangiophores of *Equisetum maximum* are rather smaller than those of the other species studied, and in the longitudinal reconstructions of the xylem of the cones it has, in many cases, been necessary slightly to exaggerate their size, in order that they should be clearly distinguishable.

THE COURSE OF THE VASCULAR STRANDS IN THE CONE.

In an analysis of the structure of the cones of *E. arvense*, *E. palustre*, and *E. limosum*, it was pointed out that the irregular network of the strands in the cone of the last species had probably arisen in the phylogeny from the more regular type of stele found in *E. arvense*. Reasons were given for believing that the more primitive type of mesh was one of the first order; that is to say a parenchymatous tract of tissue arising above a trace that has departed and one confined to a single internode. In the phylogeny meshes of a higher order seem to have arisen when the development of additional xylem at the fertile node was insufficient to close a pre-existing mesh by the union of two neighbouring strands. Any increase of xylem at the node tended, when insufficient to close a mesh, to narrow it. It was found that in cases in which a wide sweep of xylem extended uninterruptedly upwards, above a trace that had departed, throughout a whole internode, the absence of a parenchymatous mesh was due to unusual development of the woody tissues. Such cases were uncommon even in *E. arvense*; they occurred more rarely still in *E. palustre*, and none were observed in *E. limosum*. In all other cases the fact that no fresh mesh originated above a trace seemed to be due to poor development of xylem. Thus, if owing to the development of little additional xylem in the trace-bearing region a trace was given off from the edge of a vascular strand, the dying out of the xylem vertically above this trace involved, not the formation of a fresh mesh, but the sudden widening of one arising at a lower level. In cases where even less xylem was produced at the node, the parenchymatous meshes on either side of a strand persisted unnarrowed through the trace-bearing region, and no fresh mesh was formed, though the trace departed from the middle of the strand. A tendency was noticed in cones showing a reduction of the vascular system for a parenchymatous mesh to become decurrent for a little distance below and to one side of the trace above which it was considered, phylogenetically, to have arisen. Lastly, in cones showing very considerable reduction, some of the narrower strands occasionally passed through a node without giving off a trace. For a fuller discussion of the ways in which the reduction of the xylem made itself felt in *E. arvense*, *E. palustre*, and *E. limosum*, the reader is referred to pp. 668-73 and pp. 675-8 of my earlier paper on *Equisetum*.

In the cone of *Equisetum maximum*, though there are certain characters not found in the cones of the other species, the structure of which was investigated, reduction of the xylem of the cone has proceeded along the same general lines as in those species. That is to say, parenchymatous meshes tend to persist through numerous internodes, to become extended laterally, and for a little distance downwards; narrow strands also occasionally fail to give off traces at a node (cf. especially the reconstruction of



the xylem of Cone B, Pl. XIII). One expression of reduction common in the cone of *E. limosum* was not observed in the cone of *E. maximum*; in no case did two meshes originating independently become confluent owing to the dying out of the trace-bearing strand between them.

From what has been said above, it is evident that the reduction of the xylem leads to the formation of fewer meshes relatively to the size of the cone, but of meshes of higher orders. For instance, if the xylem forming a little island between two of the strands of the sixth whorl of Cone A (cf. Pl. XII) had been considerably more developed, and had fused with one or other of the strands between which it lies, the mesh between the strands giving rise to the fourteenth and fifteenth traces of this whorl would have been markedly constricted at this level; had the increase of xylem been sufficient to link the island up with both the neighbouring strands, this mesh, one of the third order arising above the whorl below, would have been converted into two meshes, the lower of the first and the upper of the second order. Thus the statistics giving the relative frequency of the meshes and the proportion among them of meshes of higher and lower orders are interesting. In the following table these statistics are given for two complete cones in each of the four species studied; under each species the cone that has the best developed xylem relatively to its size is put first. The table is constructed on a comparative basis. Thus Cone A of *Equisetum arvense*, the cone with the most developed system of xylem relatively to its size, has been taken as the unit, and the others compared to it. The first column, then, contains the actual number of parenchymatous meshes originating within the limits of each cone; the second column gives the number of meshes we should find in each cone, did these bear the same proportion to the sporangiophores as they do in Cone A of *E. arvense*. Columns 3, 4, and 5 refer respectively to the proportion of meshes of the first and second orders, of the third, fourth, fifth, and sixth orders, and of orders higher than the sixth. In all cases the traces at the extreme apex of the cone, belonging to the terminal group of incompletely differentiated sporangiophores, have been left out of account.

| Species.           | Cone. | Actual number of meshes. | Number of meshes necessary to maintain the same proportion as in Cone A of <i>E. arvense</i> . | Proportion of meshes of first and second orders. | Proportion of meshes of the third, fourth, fifth, and sixth orders. | Proportion of meshes of orders higher than the sixth. |
|--------------------|-------|--------------------------|--|--|---|---|
| <i>E. arvense</i>  | A     | 90                       | 90   | $\frac{80}{90}$                                  | $\frac{10}{90}$   | —   |
|                    | B     | 37                       | 45   | $\frac{36}{45}$                                  | $\frac{9}{45}$  | —   |
| <i>E. palustre</i> | A     | 34                       | 61   | $\frac{37}{61}$                                  | $\frac{7}{61}$  | —   |
|                    | B     | 22                       | 64   | $\frac{13}{64}$                                  | $\frac{8}{64}$  | $\frac{1}{32}$  |
| <i>E. limosum</i>  | A     | 34                       | 70   | $\frac{34}{70}$                                  | $\frac{14}{70}$   | —   |
|                    | B     | 22                       | 93   | $\frac{7}{93}$                                   | $\frac{9}{93}$  | $\frac{3}{31}$  |
| <i>E. maximum</i>  | A     | 88                       | 200  | $\frac{50}{200}$                                 | $\frac{20}{200}$  | $\frac{3}{200}$                                       |
|                    | B     | 167                      | 467  | $\frac{70}{467}$                                 | $\frac{10}{467}$  | $\frac{18}{467}$                                      |

Thus the proportion that the meshes bear to the number of sporangio-phores is by far largest in *Equisetum arvense*; the species which comes next in this matter, and therefore shows a lesser degree of reduction than the remaining species, is *E. palustre*; then follows *E. maximum*, and lastly *E. limosum*. But taking the average of the two cones, the proportion of meshes to sporangiophores differs but little in the last two species. A further test of reduction of xylem is, as already explained, afforded by the analysis of the proportion of meshes of relatively low and high orders. This test gives the same order of reduction of the species, viz. *E. arvense*, *E. palustre*, *E. maximum*, and *E. limosum*, and again *E. arvense* shows by far the least reduction, while there is very little difference between *E. maximum* and *E. limosum*.

Mention has already been made, in the description of the other cones studied, of the occasional occurrence (except in that of *E. limosum*) of considerable bands of woody tissues extending uninterruptedly above part of one whorl to the level of the next. Cones showing this character are regarded as displaying locally the greatest relative development of xylem observed in any internode. If this continuous tract of xylem above a series of traces were to involve the whole whorl instead of but one or two strands (the largest number I have observed to be involved except at the extreme apex of the cone of *E. palustre*), we should have a complete woody cylinder, devoid of parenchymatous meshes even in the internodes. Such internodal sweeps of xylem occur several times in Cone A of *E. maximum*; in one case, between whorls 3 and 4, such a sweep forms a very conspicuous feature in the reconstruction; relatively large sweeps of xylem above two or more traces that have departed are not found in Cone B of *E. maximum*—perhaps not a surprising fact, as the xylem of this cone is less well developed than that of Cone A. In both cones, however, we notice another feature bearing a superficial resemblance to this character, and leading to an increase of xylem in the internode. This character consists in the *linking up of two or three strands of xylem by the development of additional tracheides at a considerable distance below the departure of the traces*; sometimes this fusion of strands occurs but little above the level of the whorl below the one at which the connected strands give off a series of traces. These internodal sweeps of xylem must not be confused with those occurring above median traces; the latter may perhaps be a primitive character retained in places, for they occur chiefly in the cones in which the xylem of the stele has undergone less reduction. The internodal sweeps of xylem that originate at varying distances below a whorl, but never extend uninterruptedly and vertically above the traces of one whorl to the level of those of the next, seem, on comparative grounds, to constitute a fresh character in the phylogeny, leading to the increase of xylem by a method different from that found in the more primitive types

of cone. This formation of infra-nodal bands of xylem in the cone is a marked characteristic of *E. maximum*, and they must not, either, be confused with the relatively wide internodal strands, resulting from the fusion of two strands of the node below, that only give rise to a single trace at the next node. Such a strand may be as wide in the internode as a band of xylem that at the next node gives rise to two or more traces; but the strand is truly single, and its origin from two whole strands results from a local diminution of the number of members in the upper of the two whorls, while the two strands composing the band, though closely fused, have not become truly one since they give off two traces. Relatively wide single strands resulting from the fusion of two whole strands occur in *E. maximum*, e. g. trace 11 of the sixth whorl and trace 7 of the seventh whorl of Cone A (Pl. XII); they are found, too, in *E. limosum*, e. g. trace 2 of the third whorl of Cone A (Browne, p. 676, Text-fig. 5).

In the cones of the other species studied, parenchymatous meshes occasionally, though very rarely, originated or were closed—or in other words, branching or fusion of strands occurred, apparently unconnected with the departure of traces, or with an increase or decrease in the number of members of successive whorls. In Cone B these anastomoses are rather more numerous, which is not surprising if we remember that this cone is a very irregular one.

#### ALTERNATION AND SUPERPOSITION OF THE WHORLS OF THE CONE.

In *E. maximum* the alternation of the sporangiophores at the exterior of the cone is much less regular than in most of the species of the genus. Even to the naked eye considerable irregularity in the insertion of the sporangiophores is apparent, especially in the older and larger cones. Externally, Cone B afforded clear indications of the occurrence of pseudo-whorls and of the local duplication of whorls. Still, the generalization that the sporangiophores of successive whorls alternate with one another holds good for the relation obtaining in the great majority of cases.

It was recorded in the previous paper on *Equisetum* that the superposition of the meshes to the traces above which they arise was sometimes disturbed by a change in the number of traces in successive whorls. Owing to the much greater variation in the number of members in successive whorls of Cone B of *E. maximum*, and to the irregularity of their insertion, this disturbance of what may be regarded as the primitive position of the meshes (viz. one vertically above the traces) is much more widespread in this cone than in the others. But even in Cone B, in the parts in which the axial xylem is well developed and numerous fresh meshes arise, and in which there is no great irregularity in the number and position of the traces, the superposition of the meshes at their point of origin to the traces can usually be clearly seen. In Cone A this superposition



is very clear in the great majority of cases. In estimating the superposition and alternation from the longitudinal reconstructions, allowance must be made for the convergence or divergence of the imaginary lines of superposition in accordance with the decrease or increase in width of the stele.

It has already been pointed out (Browne, p. 679) that even in the species in which the sporangiophores alternated more or less regularly externally, there was no such regular alternation of the traces at their points of origin on the axial stele. When a parenchymatous mesh extends into more than two internodes the neighbouring traces cannot be accurately superposed to those of the second whorl in a downward direction, as they would be if the alternation of successive whorls were regular. In a cone, therefore, like that of *E. maximum*, in which a considerable number of meshes extend into more than two internodes, cases of regular anatomical alternation are not very common, even over a limited area. The more reduced the xylem-system the rarer such cases will be. Thus we find in Cone A a certain number of traces alternating rather regularly with the corresponding traces of the whorl below, and sometimes also with those of the whorl above. In Cone B such cases are relatively rarer. Cases of what I have elsewhere termed irregular alternation (Browne, pp. 681-2) are common both in Cone A and in Cone B. There are four forms of irregular alternation: (1) The xylem strand of an internode may be formed by the fusion of a branch of a forking strand with a whole strand of the internode below; or (2) by the fusion of two whole strands; or (3) by the forking of a single strand above the departure of a median trace, one or (4) both of the resulting strands giving off a trace. Of these modes of irregular alternation the first is by far the commonest, while the last is very rare.

More or less regular superposition of traces of successive whorls occurs commonly in cones of *E. maximum*, as it does in those of *E. limosum*, and more rarely in those of *E. palustre* and *E. arvense*. It arises in the same way as in those species. The parenchymatous meshes on either side of a strand extend upwards through several internodes, and as the strand thus pursues an isolated course through several nodes, the traces given off from it are necessarily superposed. This superposition is clearly due to reduction of the vascular system. When the meshes persisting on either side of the trace-bearing strand are narrowed by the formation of a certain amount of additional xylem at the node, the traces of successive whorls are not necessarily accurately superposed, since it is possible for successive traces to depart from different sides of the strand; but where the reduction of xylem at the node is greater, and the strand remains relatively narrow, the superposition of traces given off by it at successive whorls is necessarily more accurate. In Cone A, in which the xylem is relatively well developed, a single strand does not seem to give off more than three, or at most four, consecutive and superposed traces; but in Cone B, an isolated strand some-

times gave off as many as seven superposed traces. In Cones A and B of *E. limosum*, a species in which the stele of the cone seems to be even more reduced than in *E. maximum*, I estimate the highest number of superposed traces given off successively by a single strand as respectively four and seven, the lower number being again characteristic of the cone with the better developed xylem.

#### MEDULLARY TRACHEIDES.

Stiles has already recorded the presence of groups of medullary tracheides in a branched cone of *E. maximum* (Stiles, pp. 114-16). I have met with medullary strands or small groups of medullary tracheides in three of the seven cones of *E. maximum* that I studied, i. e. in Cones A, B, and C. In the other cones I found no traces of medullary xylem; but this by no means proved that these cones never developed medullary tracheides. The series of sections of cones other than Cones A, B, and E were not complete, and it is possible that some of these cones had medullary tracheides in parts of the cone. Moreover, except in young cones, or in the young parts of cones, the pith, or at least the central part of the pith, has perished, and small medullary strands may have perished too. Such strands would probably tend to persist somewhat longer than the soft parenchyma; an illustration of this tendency is afforded by Cone B, in which the group of medullary tracheides is embedded in a projection of pith extending for a considerable distance into the central cavity. But where there is a very large central cavity, as in the lower parts of large mature cones of *E. maximum*, medullary tracheides might well disappear, as do the tracheides developed in the position later occupied by the carinal canals, the destruction of which often begins even near the apex, where the stele remains relatively narrow. Only an examination of a considerable number of cones in which the cells of the pith are still intact can settle the question of the relative frequency of the medullary strands in the cone; but I am inclined to believe that in the species under consideration they are not uncommon.

Speaking generally, the presence of medullary tracheides in a section is easily recognized, even by the naked eye, owing to the irregular agglomeration of darkly coloured tannin-cells around them. A similar irregular concentration of large tannin-cells occurs on either side of the ring formed by the stele and all round the departing traces. Tannin-cells accompanying vascular tissue tend to have their long axes directed parallel to those of the tracheides. Besides these tannin-cells, associated with vascular tissue, isolated tannin-cells occur scattered more thinly throughout the pith and cortex. In all cases the outline of the medullary strands is shown in the longitudinal reconstructions by a broken line, black on the white and white on the black part of the diagram.

In Cone A the medullary strand, though deeply seated, was slightly

eccentric, and in the longitudinal reconstruction it has been shown on the strand on the radius of which it occurs considerably further in. The manner of its ending in a downward direction could not be ascertained, owing to the disturbance of the cells of the pith in which it was embedded; but the strand was clearly a short one. Even where it is preserved, the incipient disintegration of the central tissue of the cone makes it difficult to ascertain the details of its structure. It seems to consist of numerous tracheides mixed with unligified parenchyma, much as do the normal bundles, and of small cells pointing obliquely outwards, resembling those inside the pericycle of the normal stele, cells which we may provisionally term phloem. The orientation of this strand is therefore nearly normal.

In Cone B such medullary tracheides as have been preserved are arranged very irregularly, many running for a part of their course horizontally or obliquely across the cone. The medullary strand consists of elements similar to those of Cone A, but the phloem-like cells are distributed irregularly on all sides, while irregular endodermal markings may be seen in some of the cells outside the tracheides. In a vertical direction the extent of this medullary strand is very small, and passing upwards it dies out as two very small groups of tracheides, separated by a few phloem-like cells.

In the branched cone, C, these medullary strands are much more important. There are two of them, quite independent of one another. Throughout their course they remain at very much the same depth in the pith, but the larger one does not run quite vertically, being found now on the radius of one bundle, now on that of one of its neighbours. The lower and larger medullary strand originates about 1.5 cm. from the main apex of the cone; it extends into four internodes, and its total length is nearly 5 mm. Its branchings, the variations in its width and its course with reference to the radii of the bundles, can be seen in the longitudinal reconstruction (Text-fig. 4, p. 250). As regards radial depth, this strand occupied a position rather more than half-way out between the centre of the pith and its periphery. If we trace its course from below upwards we find that before any tracheides appear there is a little patch of about eight thick-walled cells, noticeably smaller than those of the pith. These cells are from six to fifteen times smaller than those of the pith, the variation in size occurring not in the cells of the medullary patch but in those of the pith. Outside this patch of thicker-walled small cells the thin-walled cells increase gradually in size until we reach the large cells typical of the pith. Very soon, before any tracheides appear, small phloem-like cells are to be found facing obliquely outwards; a little further up two or three tracheides identical with those of the normal bundle make their appearance. The tracheides and phloem-like cells increase in number, and the latter spread round the former until they nearly enclose them. These phloem-like cells

are, however, absent from about one-ninth of the circumference of the strand from the ninth that points straight outwards. A little later they die out, except on the inner side, and we get a medullary strand with inverted orientation, an orientation maintained throughout the rest of its course. At its inception the strand was intermediate in orientation between an inverted and a normal bundle, but nearer to the latter. Where the strand is largest, just before it branches (see Text-fig. 4 and Pl. XIV, Fig. 2), it contains, in cross-section, rather more than thirty tracheides. As the small phloem-like cells are the first to appear, so they are also the last to die out. No traces of endodermal markings were observed in the cells round this medullary strand, but in *E. maximum* they are not usually recognizable outside the normal stele of the mature cones. The other much shorter medullary strand arises just as the lower one is dying out, and is about a millimetre in length. It lies about half-way between the normal stelar bundles and the centre of the pith. It is not surrounded by a marked ring of tannin-cells, but, at the level of its origin, scattered tannin-cells are exceptionally numerous in the pith.

Though no other medullary strands were met with in Cone C, I found two or three patches of unligified cells like those that occurred above and below the medullary vascular tissue (see Pl. XIV, Fig. 2, on the reader's right); these seem to have been groups of cells that might, under conditions slightly more favourable to the development of xylem, have given rise to medullary tracheides.

#### STRUCTURE OF THE APEX OF THE CONE.

The apex of the stele of the cone of *E. maximum* resembles that of the cone of *E. palustre* rather than the apices of the steles of the cones of *E. arvense* or *E. limosum*. For in the last two species a certain number of the parenchymatous meshes of the cone persist round the strand or strands entering the terminal structure, while in *E. palustre* and in *E. maximum* all the parenchymatous meshes of the cone seem to be closed at the apex. Further, in *E. arvense* two or more strands persist into the apex of the cone, and in *E. limosum* the terminal trace forms the continuation of one of the strands of the internode below. In *E. palustre* and *E. maximum* there arises a closed ring of wood, which narrows rapidly, losing its 'pith', to form a large solid strand that passes into the apical structure. The actual diameter of this strand is greater in *E. maximum* than in *E. palustre*.

#### COURSE OF THE TRACES IN THE CORTEX OF THE CONE.

The traces entering the sporangiophores of *E. maximum* do not always pass out radially, but undergo torsion to the left or to the right. This is clearly the result of the poor development of the axial xylem at the fertile node; some tracts of parenchyma persist at this level in all cones of



*E. maximum*, and as the sporangiophores are more or less equidistant from one another, some of the incoming traces have to curve in order to attach themselves to a strand. The smaller the amount of xylem present at the fertile node the more numerous and the wider will be the parenchymatous meshes that persist; consequently, as a rule, the larger will be the number of incoming traces undergoing torsion, and the greater the degree of this torsion.

Some of the traces of the cones of *E. maximum* diverge more or less steeply downwards; others pass out horizontally, while yet others traverse the cortex in an obliquely upward direction. Statistics of the divergence of the traces of different whorls are given in the appended table for Cones A and B. Owing to the greater number of whorls in the latter cone, whorls which would be numbered alike in both cones would not occupy the same relative position. So far as was possible whorls were selected occupying the same relative positions in both cones; but the desirability of avoiding pseudo-whorls and whorls in which the cortex had been damaged, either in nature or by mounting, made it impossible to choose only whorls of exactly the same relative position. The whorls chosen were, counting from below upwards: Cone A, whorls 1, 4, 8, 12, and 17; for Cone B, whorls 1, 8, 14, 19, 26, and 27, the statistics for the last two being included under one head owing to their irregularity. These whorls have been lettered as Series A to E, from below upwards, so as to bring out the correspondence in their relative positions in both cones.

DIVERGENCE OF THE TRACES IN THE CORTEX OF CONES OF *E. MAXIMUM*.

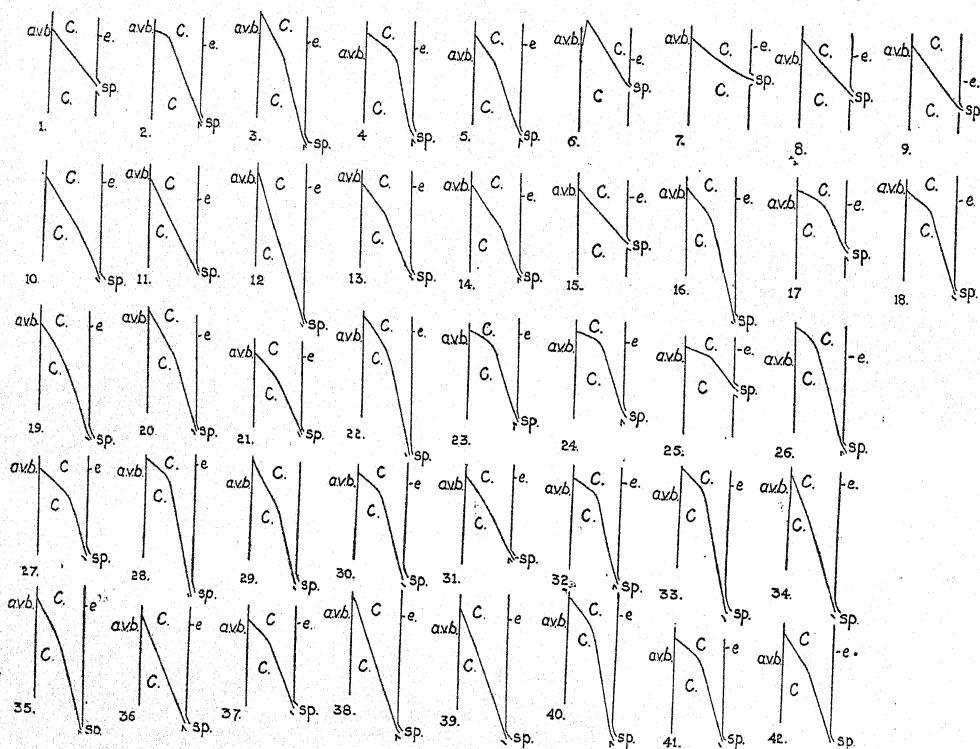
| Series. | Cone. | Extremes of divergence.                | Average divergence of traces. |
|---------|-------|--|-------------------------------|
| A       | A     | 125 $\mu$ -750 $\mu$ downwards.        | 312 $\mu$ downwards.          |
| A       | B     | 275 $\mu$ -1,500 $\mu$ downwards.      | 997.02 $\mu$ downwards.       |
| B       | A     | 200 $\mu$ upwards-175 $\mu$ downwards. | 29.5 $\mu$ downwards.         |
| B       | B     | 492 $\mu$ upwards-644 $\mu$ downwards. | 37.43 $\mu$ downwards.        |
| C       | A     | 200 $\mu$ upwards-350 $\mu$ downwards. | 32.5 $\mu$ downwards.         |
| C       | B     | 350 $\mu$ upwards-420 $\mu$ downwards. | 80.91 $\mu$ downwards.        |
| D       | A     | 300 $\mu$ upwards-75 $\mu$ downwards.  | 72.9 $\mu$ upwards.           |
| D       | B     | 140 $\mu$ upwards-294 $\mu$ downwards. | 53.63 $\mu$ downwards.        |
| E       | A     | 28.75 $\mu$ -300 $\mu$ upwards.        | 186.5 $\mu$ upwards.          |
| E       | B     | 175 $\mu$ -850 $\mu$ upwards.          | 506 $\mu$ upwards.            |

A study of this table gives rise to the following generalizations:

1. In both the cones all the traces of the lowest series, Series A, diverge downwards, and though the individual traces vary greatly in the extent of their downward divergence, the average downward divergence of this series is in Cone A rather less, and in Cone B rather more, than ten times as great as the greatest average downward divergence of the other series studied.
2. In both cones all the traces of the uppermost series, Series E, diverge upwards.
3. In the intervening series, B-D, some traces pass out upwards, some

downwards, and some horizontally in both cones. In Series B and C the average divergence of the traces is still a downward one. In both cones this average downward divergence is greater in the upper of the two whorls; this seems to be an exceptional feature, found by coincidence in both cones. In Cone A the average divergence of the traces of Series D is upwards, while in Cone B this series has an average divergence downwards.

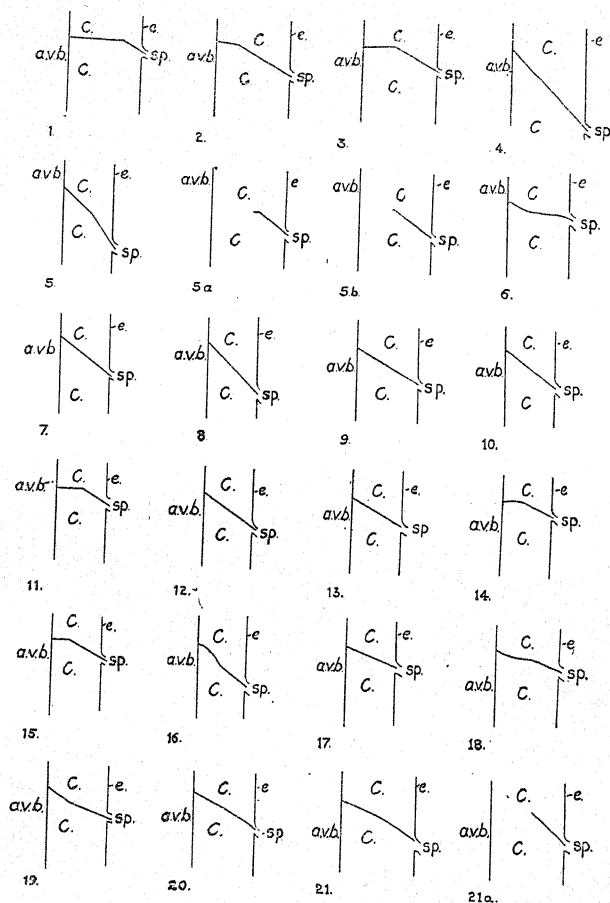
One of the most striking facts brought to light in this table is that the average downward divergence of the traces in Series A is very much greater



TEXT-FIG. 1. Divergence of traces of Series A of Cone B.  $\times 13\frac{1}{3}$ . a.v.b. = axial vascular bundle; C. = cortex; e. = epidermis; sp. = sporangiophore.

(rather more than three times as great) in Cone B than in Cone A. In mature cones of *Equisetum maximum* the sporangiophores of the lower whorls are often markedly reflexed; such reflection presumably causes a considerable 'pull' on the vascular strand passing through the cortex. It would seem very probable, therefore, that the greater extent of the downward sweep is the direct result of this 'pull'. It is true that many of the sporangiophores of Series B of Cone B were reflexed, and their traces seem to have been but little affected by the pull; but it must be remembered that the thickness of the cortex in this region of Cone B is

about twice as great as at the level of Series A (cf. p. 248) of the same cone, so that the distance at which the pull originates may well have weakened its effect a good deal. Further, the degree of reflection of the sporangiophores is itself less in Series B than in Series A. The probability that the pull of a reflexed sporangiophore is an important factor in bringing about the downward divergence of the traces is supported by the fact that in most

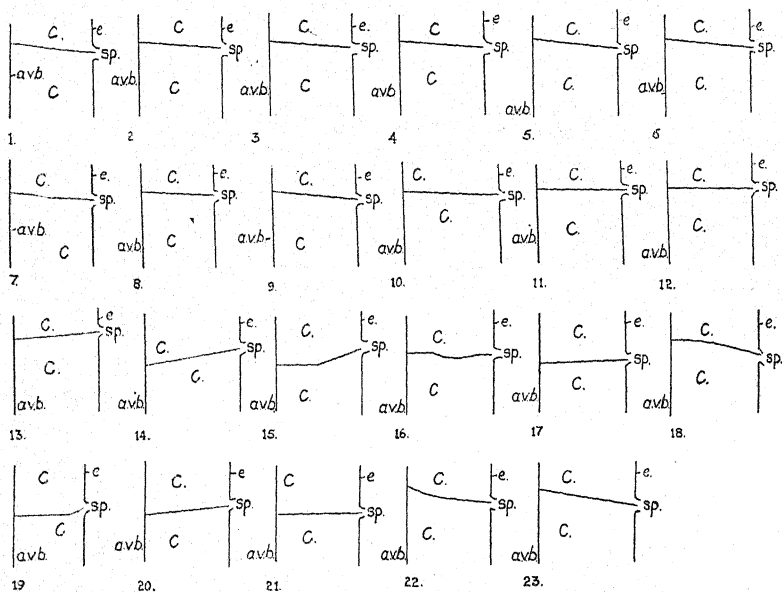


TEXT-FIG. 2. Divergence of traces of Series A of Cone A.  $\times 13\frac{1}{2}$ . Lettering as in Text-fig. 1. Note that traces 5a, 5b, and 21a die out in the cortex.

cases, though there are numerous exceptions, the downward slope of the outgoing trace is much steeper towards the periphery of the cortex, i. e. nearer to the object exerting the pull. This is especially true of Series A of Cone B, where, if there is any difference in the steepness of the course of the trace, the outer part is always the steeper (cf. Text-Figure 1).

But I do not think that the reflection of the sporangiophores is the only cause leading to the downward divergence of the traces. In the

young Cone A the reflection, supposing it to have begun, can only have been incipient, and yet the traces of the three lower series, Series A, B, and C, showed an average downward divergence. It is true that this was inconsiderable in Series B and C, in which some of the traces even diverged upwards (cf. Text-fig. 3); but in Series A all the traces diverged downwards, and the average extent of the downward sweep was  $312\mu$ , which, though it may seem a small actual distance, gives a steep angle (cf. Text-fig. 2). It is, however, clear that the lower internodes of Cone A have already elongated considerably. It seems likely, from the course of the traces, that



TEXT-FIG. 3. Divergence of traces of Series B of Cone A.  $\times 13\frac{1}{2}$ . Lettering as in Text-fig. 1.

this elongation has been greater in the inner part of the stem, i. e. at the stele, than at the exterior where the sporangiophores are inserted; in this case the point of insertion of the trace on the stele would come to occupy a higher level than the point at which the trace leaves the stem. This greater elongation of the internode in the inner part of the stem might account for those exceptional cases in which the inner part of the course of the traces is steeper than the outer. The elongation of the internode is much less as we pass upwards; for instance, even in the young Cone A it is much less considerable above Series B than above Series A. In the upper part of the cone such elongation as has taken place in the stele does not apparently exceed that which has occurred at the periphery of the stem. Among the species the cones of which I have examined, viz. *E. arvense*, *E. palustre*, *E. limosum*, and *E. maximum*, the last is the only



one in which any of the traces of the sporangiophores are markedly reflexed, and it is the species in which we get the greatest elongation of the nodes and reflection of the sporangiophores.

Thus in the lowest whorls of Cones A and C of *E. arvense* we get an average downward divergence of the traces of  $161\ \mu$  and  $157.5\ \mu$  respectively. In Cones A and B of *E. palustre* the traces of the lowest whorl have an average downward divergence of  $28\ \mu$  and  $22.75\ \mu$  respectively; in Cone C of this species, var. *polystachion*, which was younger, all the traces pass out horizontally or obliquely upwards, while in Cones A, B, and C of *E. limosum*, all young cones, the average divergence of the traces, even of the lowest whorls, is upwards. In the cones of all these species, except Cone C of *E. arvense* (which is older and larger than Cone A of this species), the traces of the second whorls passed out horizontally or with an average upward divergence. In Cone C of *E. arvense* the average divergence of the traces of the second whorl was a downward one of  $14.09\ \mu$  (for details as to the age and size of these cones the reader is referred to my earlier paper).

These figures are significant. Yet, though the descending course of certain traces seems to be chiefly due to the pull exerted by reflexed sporangiophores and to the greater elongation of the stele than of the outer tissues of the stem, it is possible that a slightly downward course of the traces of the lowest whorls, even at their origin, is a characteristic of the cones of *E. maximum*, for I was unable to obtain a cone young enough to be sure that a very slight downward curve of the traces of the lowest whorl did not exist at the moment of lignification of the tracheides of the trace.

#### ABNORMAL BEHAVIOUR OF SOME OF THE TRACES IN THE FIRST AND TWELFTH WHORLS OF CONE A.

In the longitudinal reconstruction of the axial xylem of Cone A Plate XII may be seen four white crosses, three in the lowest and one in the twelfth whorl. These are to be found on strands opposite which, at that level, incoming traces died out in the cortex. Of the three abortive traces of the lowest whorl two occur in the neighbourhood of the sixth and seventh traces of the longitudinal reconstruction. These two additional traces penetrate into the cortex of the stem from the two unusually large sporangiophores supplied by the sixth and seventh traces respectively. Within the sporangiophores they are of quite normal development; indeed, the abortive trace lying near the seventh trace is, throughout its course, rather larger than the latter. Further, the large sporangiophore supplied by the fifth trace possesses another well-developed trace not marked in the reconstruction, as it dies out at the point of junction of sporangiophore and axis. In the third case an abnormal trace between the twenty-first and first traces

of the lowest whorl enters the cortex from a rather small but quite normal sporangiophore, possessing spores normal in appearance, and dies out opposite to but without reaching a strand that gives rise to no trace in this whorl. All these abortive traces of the lowest whorl die out rather less than half-way through the cortex. In the twelfth whorl the sporangiophore penetrated by the second trace in the reconstruction possesses another trace that dies out about one-third of the way through the cortex; in the sporangiophore this trace lies close to the second trace, but passing inwards and downwards into the axis it undergoes torsion to the reader's left until, when it dies out, the distance between it and the second trace is twice what it was midway in the sporangiophore. This bifascicular sporangiophore seems not merely to be unusually large, as were the bifascicular sporangiophores with one abortive trace of the lowest whorl, but to represent two fused sporangiophores. This is borne out by its greater size relatively to its neighbours, by the marked divergence of the two traces as they pass inwards, and by the fact that independent parenchymatous meshes arise above each of them.

In Cone C of *E. limosum* I observed one case of one of the two strands of a bifascicular sporangiophore dying out in the cortex. This strand was relatively small, and its xylem died out almost in contact with the axial xylem, while it appeared as though the phloem of the trace actually joined on to that of the axial stele.

It would be natural to suppose that the dying out of the traces in the cortex, which is so marked a feature of the lowest whorl of Cone A, might result from a diminution in the size of the stele relatively to that of the area of distribution of the sporangiophores, viz. to the circumference of the axis of the cone. But the reverse is the case; from the appended table it can be seen that compared with the circumference of the stem the stele is larger in the lowest whorl.

| <i>Number of whorl.</i> | <i>Circumference of stele.</i> | <i>Circumference of axis.</i> |
|-------------------------|--------------------------------|-------------------------------|
| 1                       | 14.25 mm.                      | 17.85 mm.                     |
| 4                       | 13.6 mm.                       | 18.91 mm.                     |
| 8                       | 12 mm.                         | 17.56 mm.                     |
| 12                      | 8 mm.                          | 13.07 mm.                     |
| 17                      | 1.4 mm.                        | 5.85 mm.                      |

#### THICKNESS OF THE CORTEX IN THE CONE.

The thickness of the cortex varies somewhat at the same level; it varies very greatly at different levels. It is not only relatively but actually narrower in the lowest whorls of the cone. The thickness of the cortex and that of the stele are given in the appended table for five selected whorls of Cone A, numbered from below upwards. This narrowness of the cortex at the base of the cone was a marked feature of all cones of *E. maximum* that I examined.

| Number of<br>the whorl. | Average radius<br>of the cortex. | Diameter of stele. |
|-------------------------|----------------------------------|--------------------|
| 1                       | 0.6 mm.                          | 4.73 mm.           |
| 4                       | 0.88 mm.                         | 4.53 mm.           |
| 8                       | 0.92 mm.                         | 4 mm.              |
| 12                      | 0.84 mm.                         | 2.6 mm.            |
| 17                      | 0.72 mm.                         | 1.46 mm.           |

#### BRANCHING CONE.

Branching cones of *E. maximum* have been recorded by Stiles and Milde (Milde, p. 250), but not by Duval Jouve except as a great rarity, due to mutilation (Duval Jouve, p. 154). The abnormalities of Cone C, its branching and medullary strands, are confined to its upper part (cf. Text-fig. 4). At a distance of about 10 or 11 mm. from the apex of the cone (the latter had not fully elongated) it branches for the first time; a second branch is given off about a millimetre above the first, and a third one just above the second. The first and second branches are very much smaller than the parent cone, while the third is a little more than three-quarters of its size.

As may be seen from the longitudinal reconstruction of the xylem of this part of Cone C, the vascular tissue of the lowest branch is of considerable extent where it is inserted on the axis, and the elements that are passing out are cut very obliquely in transverse sections of the main cone. The xylem of the branch forms a nearly closed ring inserted very obliquely on three of the bundles of the axis, the two outer ones of the series contributing relatively few tracheides. The vascular tissues of the branch are given off by constriction of a loop of stelar tissue, so that no gap is left in the main stele. After the vascular tissue of one side of the branch has become free from the parent stele, but while the vascular elements of the other side are still in connexion with those of the parent axis, a trace goes off from the point of insertion of the vascular system of the branch on that of the main axis. This enters a sporangiophore situated at the junction of branch and cone. Soon afterwards the vascular tissue of the branch becomes finally free, constituting a slightly incomplete disto-proximally elongated ring that passes obliquely outwards. At the proximal end the ring of xylem is thicker, and from this region a trace is eventually nipped off in a direction pointing radially inwards; but bending sharply round backwards it passes out, somewhat higher up, as a second trace, into the sporangiophore already mentioned as inserted at the point of junction of the branch and main cones. A little higher up, while the stele of the branch is diverging very obliquely, a trace departs for another sporangiophore, one situated at the other point of junction of the branch with the main cone; this sporangiophore, however, belongs definitely to the branch cone, and, like the sporangiophores of the latter, is somewhat smaller than those of the main cone at the same level. This branch cone pursues throughout its



TEXT-FIG. 4. Longitudinal reconstruction of the xylem of the region of branching of Cone C. x 20. Axial xylem black, traces and parenchyma white; insertion of branch steles dotted; outline of medullary strands in broken lines. The xylem of the stele of the cone has been hypothetically cut and laid flat.

existence a course remarkably oblique with reference to that of the main cone. Two more traces depart from the branch stele away from the parent cone; as it passes upwards and outwards the annular stele decreases considerably in size, and the xylem, closing on the side in which it was open, opens very slightly on the other side, to close again almost at once. After the departure of the two last-mentioned traces the stele condenses and becomes a large solid strand running into the apex of the branch cone and spreading out in the latter, which consists of a relatively large mass of tissue in which separate sporangiophores are not differentiated.

It may be seen in the longitudinal reconstruction that on one side of the branch—in the direction in which the branch cone passes obliquely outwards—there are in the stele of the main cone two parenchymatous meshes not subtended by traces. The traces which we should expect to occur below these meshes are not developed, since, owing to exigencies of space, the sporangiophores they would naturally supply are not formed.

The stele of the second branch of the cone is also given off from the parent cone without the formation of a gap, the parenchymatous mesh which may be seen above the end of the stelar branch clearly belonging, to judge from its size and position, to the trace found at the edge of the branch, but belonging to the main cone. The gradual formation of a loop of vascular tissue begins some time before it is finally given off by constriction as a branch stele, and three traces are given off from this loop before it becomes free, two on its distal side and one near one of its flanks. These pass upwards and supply the sporangiophores of the lowest whorl; the latter are inserted on the side of the branch cone furthest from the parent cone; in this whorl there are no sporangiophores on the adaxial side of the branch cone, presumably owing to exigencies of space. Three parenchymatous meshes appeared above this lowest incomplete whorl, two of them subtended by traces that have departed. The second and last distinct whorl of this branch of the cone was complete, but the stele had become narrower, and there were only four sporangiophores arranged more or less regularly round the axis. The traces for these sporangiophores depart from somewhat different levels, and pursue upward courses of varying steepness. Above this whorl the apex of the cone was undifferentiated into distinct sporangiophores. Unfortunately the sections of the extreme apex were lost in mounting, but a sufficient number were preserved to show that in all probability it followed the type normal for this species.

The third case of branching is somewhat different. It is apparently an example of unequal dichotomy, resulting from the gradual constriction of the stele near, but not quite at the middle. The slightly larger stele remains terminal in position, and at the level of separation of the two steles consists of seven bundles in an internodal condition. The smaller, or branch stele, diverges at a much more acute angle than did the steles of

the other branches, and when, after the separation of the branch stele, the branch as a whole separates from the main axis, its vascular tissues assume a course nearly, but not quite, parallel with those of the main cone. At the point of separation of the two steles that of the branch cone consists of two large unequal bands of xylem. At a level at which the separate steles are still connected by a wide band of parenchyma the band of tracheides on the side of the branch stele near the larger stele gives off two traces. These curve round in divergent directions, and higher up enter the two sporangiophores nearest the terminal cone; these sporangiophores are inserted on the branch cone in an adaxially lateral position with reference to the main cone. The traces are not given off in clearly marked whorls, and nearly every section passes through traces in different stages of their course or origin. This is true of many of the apices of the cones of this species. Nevertheless, from the size of the stele and the height of the branch it is clear that over one-third of its circumference this branch cone bears two whorls, while over the other two-thirds only one whorl is developed, and that the upper one of the two. The reason for the absence in the lower whorl of sporangiophores on one of the flanks of this third branch cone is that the latter is here very close to the second branch of the cone, lying just below and to one side of it, and to the parent cone. The lowest imperfect whorl seems to consist of five sporangiophores, one bifascicular, while the upper complete one, given off when the stele has become narrower, consists of six sporangiophores. Most of the traces of this branch pursue a course that has undergone a certain amount of torsion to right or to left. Above these two whorls we find, in the branch cone, a rather large strand resulting from the condensation of the stele, which, as in other cases, runs through the terminal structure of the cone, widening out before it dies out.

#### THE FERTILE STEM BELOW CONE D.

Cone D, kindly given me by Mr. E. M. Cutting, M.A., was a very large mature cone, somewhat withered before it was pickled. The region transitional from the cone to fertile stem showed some abnormalities; over a part of the circumference of the axis the annulus appeared to be replaced by scattered sporangiophores (cf. Duval-Jouve, p. 175). There seemed to be the usual supra-annular anastomoses of the vascular strands, but the tissues in this part of the stem had collapsed too much, before it came into my hands, for it to be possible accurately to follow the course of the strands. In other respects the lower part of the cone seemed to resemble the corresponding region in other cones of *E. maximum*; i. e. the bundles contained relatively few, poorly lignified tracheides, and the very narrow cortex was traversed by traces inserted at somewhat various levels and pursuing a steeply downward course.

In the sterile stems of this species generally a varying number of

branches, usually a number equal to the number of traces and bundles, is given off in the region of the node. The incoming stele of the branch forms a continuous ring or cylinder, joining on obliquely to the adjacent ends of two neighbouring bundles (Pfitzer, pp. 329-30). I have sometimes observed similar but arrested branches in sections of fertile stems of *E. maximum*. In some cases these branches of the fertile stem are fully developed, as recorded by Milde in the variety *frondescens* (A. Br. 1844) of *E. maximum* (Milde, p. 249). Though the branches I observed never reached the periphery of the stem, their vascular system bore the same relation to that of the main stem as did the vascular system of the fully developed branches to the main stele of the vegetative stems, and there seems no reason to doubt that this is true of the branches of the fertile stem of the variety *frondescens*. The fertile stem bearing Cone D also bore two abortive branches, but the relations of their vascular tissue to that of the stem were very different. Both were inserted on the internode, or what would normally be an internodal part, between the annulus and the upper vegetative whorl. These two branches never become really free from the stem, but form a projection or hump over part of the circumference of the latter. Both these projections or abortive branches contain a vascular system which is connected with that of the main stem towards the upper end of the projection. In other words, the free end and organic apex of the vascular system of the hump projects downwards; but, as the parenchymatous tissue in which it is embedded is congenitally united with the stem, it follows that the lower cells of the hump or abortive branch must have been produced first. The stem being quite mature when cut, it is impossible to say whether the lignification of the branch stele proceeded in a downward direction, viz. from the point of insertion of the branch stele on the main stele; or upwards until eventually a junction was effected between the steles of branch and stem; or whether lignification began in the middle region of the hump and proceeded both upwards and downwards. One effect of the downward direction of the branch steles is that the stele of the main stem is larger and contains more bundles above than below their insertion.

The larger and abortive branch contains in its middle region, that in which the stele is most developed, eleven bundles. Of these the five outermost bundles, forming an abaxial arc, open adaxially, constitute the continuation, in a steeply downward direction, of five adjacent bundles of the main stem. These bundles pass out in their entirety, but as they are constricted off as a loop of distinct bundles they leave no gap in the main stele. The remaining six adaxial bundles of the branch stele are derived from four other bundles of the main stem. These four bundles do not themselves pass out, but each bundle divides in a plane approximately coinciding with a line laterally across the bundle as seen in transverse section. The outer series of bundles then passes out, diverging slightly

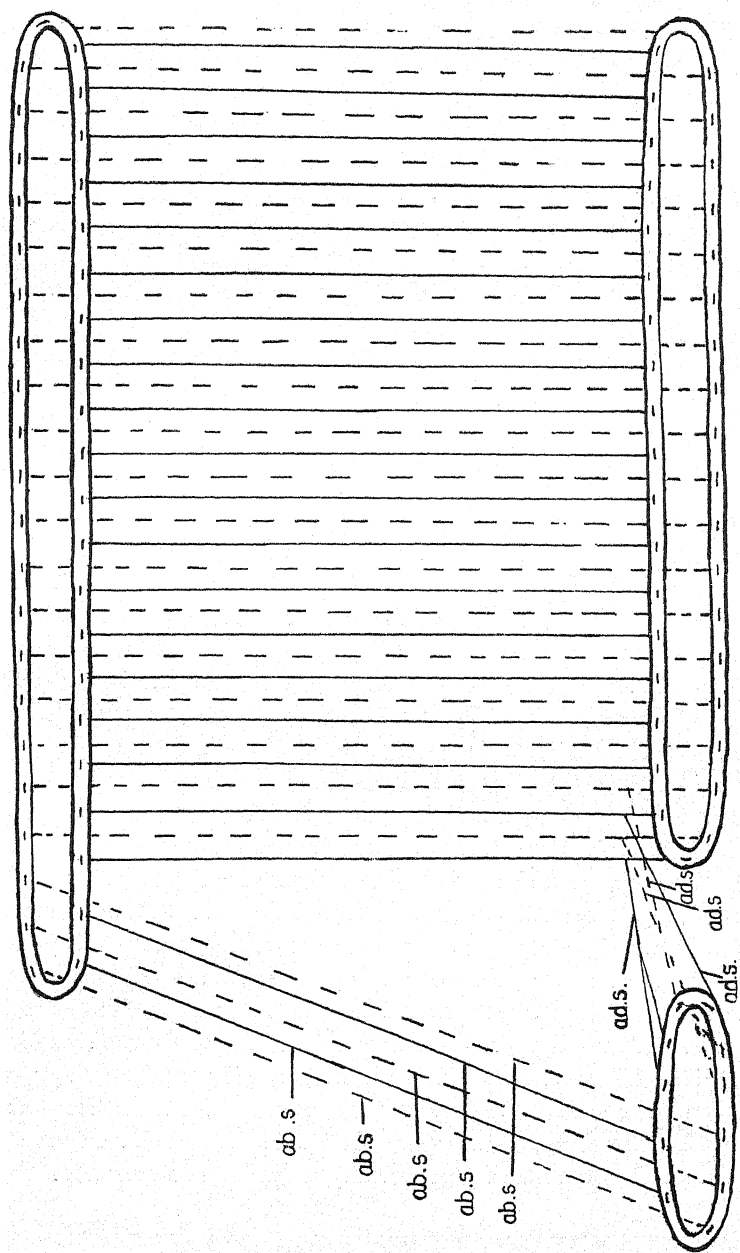
downwards (but much less steeply than do the bundles of the abaxial arc), and the two bundles at each end of this series divide again in a plane nearly coinciding with a line drawn radially through these bundles (the latter are by now arranged nearly back to back, but at a certain distance from the bundles of the stem that gave rise to them by division). The resulting series of six bundles forms the adaxial arc of the branch stele. Above the insertion of these adaxial strands the parenchymatous hump only contains the abaxial series open adaxially; after the adaxial bundles have taken their place in the circle of the branch stele no traces remain of the distinction in origin between the bundles of the arcs. Lower down, at the departure of the adaxial strands, there is also no break in the stele of the stem, since the bundles of the branch stele arise by the forking of bundles, and the inner strands resulting from this forking pursue a course vertically downwards. The main stele, which above the departure of the abaxial bundles of the branch stele had forty-three bundles, has below their departure thirty-eight, a number which is not affected by the departure of the adaxial bundles. The relations between the strands of the main stele and the vascular system of the larger abortive branch are diagrammatically represented in Text-fig. 5.

The stele of the lower and smaller abortive branch consists, when it reaches its greatest development, of six bundles. The two outermost bundles of this branch have passed out in their entirety from the main stele, while the four adaxial ones arise by the division of the two bundles of the central cylinder, and by a second division of two bundles of the series that arose by the first division. The planes of these divisions are respectively similar to the first and second divisions, giving rise to the adaxial bundles of the larger abortive branch. Below the insertion of the two abaxial bundles of the smaller branch and by their departure the number of bundles in the main stem is reduced to thirty-six. This is also the number of traces in the uppermost leaf-whorl, and of the bundles in the internode below it.

In the smaller abortive branch the vertical distance between the insertion of abaxial and adaxial bundles on the main stele is only  $980\ \mu$ , whereas in the larger abortive branch the vertical distance between the insertion of these two series of bundles is not less than 9.7 mm., possibly considerably more. The distortion of the stem in this region led to the breaking and tearing of these tissues, so that it is impossible to determine the exact distance between the departure of the abaxial and that of the adaxial bundles of the branch from the bundles of the axis. In the diagram the smallest possible distance has been adopted.

In both abortive branches, as we pass downwards in the parenchymatous projection, after the entry of the adaxial bundles into the branch stele the latter condenses, and the bundles fuse and die out, still running obliquely downwards. This occurs before we reach the lower limit of the





TEXT-FIG. 5.  $\times 95$ . Reconstruction of the stele of the stem below Cone D and of the larger abortive branch. *ab.s.* = abaxial strands of the branch stele; *ad.s.* = adaxial ditto. The continuous lines represent the bundles on the near side of the stele and their branches; the dotted lines those of the back of the stele and their branches, the stele being hypothetically transparent to show their course.

parenchymatous humps. In the smaller hump the bundles never form a continuous circle, and there is a gap adaxially, involving about one quarter of the circumference. At the level of insertion of the adaxial strands of this smaller branch on the two bundles of the main stele the latter bundles become temporarily united to one another by a narrow band of reticulate tracheides, resembling the elements of the nodal or supranodal xylem. All the other bundles of the main stem as well as the abaxial bundles of the branch stele remain throughout in a typically internodal condition, while the daughter and granddaughter bundles of the strands that showed reticulate tracheides—viz. the adaxial bundles of the branch stele—become typically internodal soon after entering the parenchymatous hump. As regards the larger abortive branch its abaxial bundles and all the bundles of the main stem at the level of insertion of abaxial and adaxial bundles retain a typically internodal structure. But the three adaxial bundles of the branch stele themselves develop a few reticulate tracheides; these soon die out and are never sufficiently numerous to unite any of the bundles laterally to one another.

The four strands that are given off by the main stem to form, after subsequent division of two of their number, the six adaxial bundles of the larger abortive branch give off, before this second division, four traces pointing more or less straight adaxially towards the bundles of the main stem. At a lower level these enter a sheath inserted at the lower point of junction of stem and hump. This sheath surrounds the adaxial half of the hump, and is in reality inserted obliquely in the angle between it and the main stem. Its connexion with the latter ceases higher up than (i. e. owing to the inversion of the branch, before) its connexion with the hump, and as its traces are derived from the bundles of the branch stele it may be regarded as belonging to the abortive branch, though it is curious that it should occur not on the free but on the adaxial side of the latter. A similar, smaller sheath, containing three bundles, occurs in connexion with the small abortive branch, and is here found, as we should expect, on the abaxial side of the hump. Its traces, however, pass out obliquely from the adaxial bundles, before these have divided a second time, and whilst they are therefore two in number. Both bundles give off traces at approximately the same level, and these diverge into the sheath from different sides of the hump; later (i. e. lower) one of the two gives off another trace. All these traces actually pass steeply downwards and outwards, but in view of the inversion of the abortive branch they are passing organically upwards and outwards.

#### NORMAL NODES OF THE FERTILE AND STERILE STEMS.

The uppermost vegetative whorl of the stem bearing Cone D was quite normal. There were thirty-six bundles in the internode above and below it, and thirty-six traces were given off. Vertically above the

points of departure of these traces we find well-developed reticulate tracheides, and it is, as pointed out by Jeffrey (Jeffrey, 2), only higher up, when the nodal tracheides die out, that the parenchymatous meshes make their appearance.

A node of the sterile stem was cut serially for purposes of comparison, and afforded an example of increase of bundles in successive internodes. In the internode there were twenty-five bundles, showing slight variation in size and irregularity in distribution, but clearly equivalent to one another. Twenty-five traces were given off. In the upper internode there were twenty-six bundles, two of them being very close together. This couple of bundles, of which one was much smaller than the other, results from the forking of a strand at its very origin, i. e. at the node. In other words, we get at this level the formation of a parenchymatous mesh independent of the departure of a trace. Such fission seems to be the usual mode of increase of the bundle in successive internodes. Similar cases leading to increase in the number of bundles were met with in the fertile stems and axes of cones, not only in this species, but in *E. arvense* (Browne, p. 679). The disturbance of the alternation of the bundles of successive internodes is narrowly restricted to the strands involved in the forking. But except for such irregularities, due to the increase or decrease of the number of bundles in successive internodes, the parenchymatous meshes of the stems of *Equisetum maximum*, fertile and sterile alike, seem to be disposed very regularly above the traces, so that the bundles and therefore the carinal canals alternate very regularly from one internode to the next. I am consequently unable to understand Sykes's longitudinal reconstruction of a node of *E. maximum* (Sykes, p. 130). Here two carinal canals belonging to successive internodes are drawn as superposed. The structure of the nodal tracheides of this specimen was exceptional, and exceptional superposition of the bundles at the node may have occurred also; but as no mention of so remarkable a course of the bundles is made, it is possible that the superposition of the canals in the diagram is due to a slip of the draughtsman's pencil. Jeffrey, however, figures a case of non-alternation of the bundles at the nodes of *E. hiemale*, and states that in this species such a phenomenon is not rare (Jeffrey, 1, p. 175). To return to the small bundle, the latter is at first very close to its sister bundle, and has no carinal group of tracheides. The two lateral groups of metaxylem characteristic of the Equisetaceous bundle are, however, developed. These have been torn in part, and are very poorly lignified; sometimes, indeed, the lignification appears to be absent for a section or two. But this tearing and poor lignification of the xylem in the internodes is quite common in stems of *Equisetum*. As we pass up the internode a small carinal canal appears, but no clearly lignified elements were developed below its origin. Those which presumably occurred in the region now occupied by the carinal canal have, of

course, disappeared too. Otherwise the small bundle was perfectly normal, and as we pass upwards in the internode it moves gradually further and further away from its sister bundle, increasing a little in size. Though the series of sections did not extend very far up into the internode it seems clear that the bundle eventually became an independent trace-bearing strand.

PHYLOGENY OF THE CONE OF *E. MAXIMUM* AND ITS BEARING  
ON THE FOSSIL CONES OF THE EQUISETALES.

It has already been pointed out that the xylem in the axis of the cone of *E. maximum* is less well developed relatively to its size than that in the axes of the cones of *E. arvense* and *E. palustre*. If we were to construct a series showing progressive reduction of the vascular system of the axes of the cones of the four species studied, the order of the species would be: *E. arvense*, *E. palustre*, *E. maximum*, and *E. limosum*. The difference in the degree of reduction of the xylem relatively to the size of the stele is very slight between the last two species. The reduction of the xylem system seems to express itself in the phylogeny by the persistence of parenchymatous meshes upwards through more than one internode, by their extension laterally and for a little distance downwards, by the presence of a considerable number of unlignified parenchymatous cells mingled with the tracheides, and by the poor lignification of the latter. On the other hand, the occasional widening and consequent fusion of two or more bundles considerably below the level of departure of the traces gives rise to a local increase of xylem. This appears not to be a palingenetic character, since it is not found in the cones of the species that have, relatively to their size, a better developed vascular system.

In the case of existing Equisetaceae there is every reason to believe that they are forms derived by reduction from plants resembling the larger mesozoic *Equisetites*. It would seem, however, from a recent paper of Compter's on impressions from the Keuper that some specimens of *Equisetites* had cones resembling those of the Calamariae rather than those of the Equisetaceae, since the fertile whorls seem to have been separated by sterile ones (Compter). Dr. Scott states that on the whole the dimensions of the mesozoic Equisetaceae decrease as we approach the later horizons (Scott, p. 83). It would therefore seem, *a priori*, natural to look upon the larger species as likely to show the more primitive structure. Of all the cones hitherto studied those of *E. maximum* are by far the largest. But we have seen that the axial steles of these cones are among the more irregular and, on the view advanced in these papers, the more reduced. The possibility at once suggests itself that this irregularity of the vascular system of the cone may, since it occurs in the larger cones, be a primitive character and not due, as I have maintained, to the extension of parenchymatous meshes upwards, downwards, and laterally. I still hold that

a comparative study of the anatomy leads to the conclusion that in all probability the more regular type of stele, possessing more xylem relatively to its size, is the more primitive form, and in the following paragraphs I shall bring forward certain considerations that seem to me to detract greatly from the force of the suggestion that the larger cones are presumably anatomically the more primitive. At the same time I want frankly to admit that though, to my mind, these considerations weaken the force of this objection, they do not entirely remove it.

Accepting the view that the living Equisetaceae have undergone reduction in size, it seems probable that their vascular system has also undergone reduction. This assumption is supported by the remarkably small amount of xylem found in the internodes of the rhizome, aerial stems, and branches, and by the palustrine habit of a large number of the species of the genus. But we have no right to assume that all parts of the plant have been equally affected by reduction. For instance, we know from Halle's study of the mesozoic impressions of the Equisetales of Sweden that in some species grouped by him under the genus *Neocalamites*, the bundles of the stem only gave off traces at alternate nodes or even less frequently (Halle, p. 6). If, as seems likely, we are here dealing with a case of reduction in the phylogeny of the number of leaves, it is clear that the reduction in the number of the bundles of the axial stele has proceeded at most only relatively half as fast as the reduction in number of traces and leaves. It does not follow that the leaves were twice as far apart as in the hypothetical ancestor in which the bundles gave off traces at every node, for along with this change a decrease in thickness of the cortex, and therefore in the circumference of the stem, may well have taken place. As regards the cones, it would seem that their greater or less height could have little or no influence on the closure or persistence of the meshes at the node. *But if the reduction in width of the stele did not keep pace with the reduction of xylem at the neighbourhood of the node, the direct and immediate effect would be for some of the meshes to persist into the internode above.* I think that this is the explanation of the greater reduction of the vascular system in the cones of the species of *Equisetum* that have relatively wide steles. Thus the cones of *E. palustre* and *E. limosum* are, on an average, much of the same height, but the reduced steles of the latter species are, on an average, about twice as wide as those of the former. Then, too, the most reduced axial vascular cylinders were found in the cones of the species that had the widest steles, namely *E. maximum* and *E. limosum*. Again, within the limits of single species, Cone C of *E. arvense* and Cones B of *E. maximum* and *E. limosum* have respectively wider steles and a more reduced system of xylem than the respective Cones A of these species. In *E. palustre*, excluding var. *polystachion*, in which all parts of the cone seem to have undergone an equal degree of reduction, the cones examined had steles of much the same width,

Of course the width of the stele is not the only, nor the principal factor causing reduction of the vascular system; it is, for instance, characteristic of *E. arvense* to have cones containing more axial xylem, both actually and relatively to their size, than those of *E. palustre*, though the steles of the former species are usually much wider than those of the latter. I am inclined to believe that the relatively large amount of xylem at the apices of the cones of *E. palustre* (Browne, p. 683) is due to the considerable reduction in width of the stele in this region.

The downward curvature of the traces of the sporangiophores in the lower part of the cone of *E. maximum* recalls the downward curvature of the sporangiophore traces described by Hickling in *Palaeostachya vera* (Hickling, 1, pp. 375-6). This similarity seems to me no indication of a close affinity, for, so far as we can judge, the sporangiophores of *Equisetum* appear to be whole appendages, while those of *Palaeostachya* seem to be morphologically lobes of a fertile leaf. It is worth noting that in the larger, very irregular Cone B of *E. maximum*, in which the whorls were often locally duplicated, there was very rarely any indication of any dorso-ventral duplication of any individual trace. Even the analogy is not a very close one. In *Palaeostachya* the traces of the sporangiophores ascend steeply upwards through about half the internode, and are then sharply reflexed downwards. The angle thus formed between the outer and inner parts of the course of the trace is filled by sclerized tissue, and the latter also lines the underneath of the trace when, at the extreme periphery of its course, it passes out horizontally and enters the sporangiophore. In the lower whorls of mature cones of *E. maximum* it is common for the downward course of the trace to be steeper in its outer than in its inner part (Text-fig. 1); but except for the course of the trace represented diagrammatically in Text-fig. 1. 6, apparently an exceptional course affording no good basis for generalization, I have never, in these lower whorls, observed traces directed upwards even in the inner part of their course. This seems to be due to the fact that in the lower whorls the sporangiophores originate as structures at right angles to the axis and do not point obliquely upwards.

Were a reflexed sporangiophore to exert a pull on the trace of a cone in which the sclerenchyma was distributed as in *Palaeostachya vera*, it would only affect the sporangiophore trace in the outer part of its course, since the buttress of sclerized tissue outside the inner part would be far too strong to allow this part of the trace to respond directly to the downward pull. But it would seem that no such reflection of sporangiophores can account for the downward sweep of the sporangiophore trace of *Palaeostachya*, for in Hickling's diagram the sporangiophores are directed obliquely upwards, a condition also observable in Fig. 21 of his Plate XXXIII.

Though other figures of *Palaeostachya*, notably one of *P. gracilis*, Ren., reproduced by Solms-Laubach from Zittel's text-book (Solms, p. 332) and again by Jongmans (Jongmans, p. 322), show shorter and more nearly horizontal sporangiophores, it would seem that even in old age the sporangiophores could hardly have been much reflexed as they would soon have come to rest on the surface of the subtending bract. From the latter they are only separated by the thickness of the sclerized band and by the additional space left by the slight downward 'bowing' of the bract at its base.

To judge from Hickling's figure the actual distance in a downward direction traversed by the sporangiophore trace of *Palaeostachya vera* seems to have been rather less than 1.3 mm., while in extreme cases in *E. maximum* it is as much as 1.5 mm. of the part. Cone B, however, in which this height was recorded, seems to have had a stelar diameter of more than twice that of the *Palaeostachya* described by Hickling; the sporangiophores of the latter genus seem, too, to have been smaller and provided with fewer sporangia than those of *E. maximum*.

Hickling mentions the fact that though *Palaeostachya* shows no regular alternation of the bundles in successive internodes of the stem, occasional irregular communications seem to have occurred between adjacent bundles. As pointed out in the previous pages an irregular alternation of the bundles of successive nodes occurs in the cone of *Equisetum*, where it is due to a reduction of xylem at the nodes. It would be interesting to know if the same explanation applied in the case of *P. vera*.

The cones of *E. maximum* also recall those of *Calamostachys Binneyana* in one point. In both cones some of the traces have undergone torsion while passing through the cortex (Hickling, 2, p. 10). In *Calamostachys*, however, it is not the traces of the sporangiophores but those of the bracts that pursue a curved course, and the curvature is throughout a whorl in the same direction. Hickling regards this curvature as 'the only possible explanation of a well-known difficulty in the morphology of this cone—viz. the combination of superposed sporangiophores and non-alternating bundles with alternating bracts' (Hickling, 2, p. 10). In *E. maximum* the torsion, which may be to right or to left, results from the fact that the persistence of relatively wide tracts of parenchyma at the node causes the incoming traces to curve in order to attach themselves to a bundle.

#### SUMMARY.

1. The xylem of the axis of the cone of this species is more reduced, relatively to its size, than that of the axes of the cones of *E. arvense* and *E. palustre*, but slightly less so than that of the corresponding region in *E. limosum*.

2. The cones with wider steles have, on the whole, a more reduced vascular system, presumably because the reduction in width of the stele has not kept pace with the reduction in the number of elements lignified.

3. The reduction of xylem is manifested, as in the other species studied, by the persistence of parenchymatous meshes upwards, and by their extension, phylogenetically speaking, laterally and downwards. This leads to great irregularities, especially in the larger cones; in the latter these irregularities take the form of local duplication of the whorls, and in extreme cases of the development of pseudo-whorls.

4. Owing to the persistence of meshes on either side of a strand through more than two internodes, cases of superposition of traces at their origin are relatively common, especially in the cones with relatively more reduced vascular system.

5. Another effect of reduction of the vascular system is the presence of unlignified parenchymatous cells between the tracheides, and the poor lignification of the latter, especially in the lower part of the cone.

6. One character, apparently relatively new in the phylogeny, leading locally to increase of xylem, is the tendency for two or more strands to become united by the production of additional tracheides at a considerable distance below the departure of the traces.

7. Groups of medullary tracheides seem to be not uncommon in the cones of this species.

8. The traces of the lower whorls of sporangiophores often diverge downwards; this is especially common in the mature cones, and is probably chiefly due to the pull exerted by reflexed sporangiophores, and to the unequal elongation of the inner and peripheral tissues of the axis. Though the analogy with *Palaeostachya* is suggestive, the sporangiophores appear to be morphologically distinct in the two genera. The analogy in the course of the traces of the sporangiophores of *E. maximum* and *Palaeostachya* is not a close one, and their downward sweep is probably not due mainly to the same causes.

9. An abnormal branching of the cone is described, as are also exceptional cases of the dying out of incoming traces in the cortex. An example of abnormal abortive branching of the vegetative part of the fertile stem was also met with.

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EXPLANATION OF PLATES XII-XIV.

Illustrating Lady Isabel Browne's paper on *Equisetum*.

PLATE XII.

Longitudinal reconstruction of the xylem of Cone A of *E. maximum*. The xylem of the cone stele has been hypothetically cut and laid out flat. Axial xylem, black; sporangiophore traces and parenchyma, white; outline of medullary strand, dotted. In this and in the reconstruction of the xylem of Cone B the size of the traces has been slightly exaggerated for the sake of clearness.  $\times$  circa 20.

PLATE XIII.

Longitudinal reconstruction of the xylem of Cone B of *E. maximum*. Axial xylem, black; sporangiophore traces and parenchyma, white; outline of medullary strand, dotted.  $\times$  circa 20.

PLATE XIV.

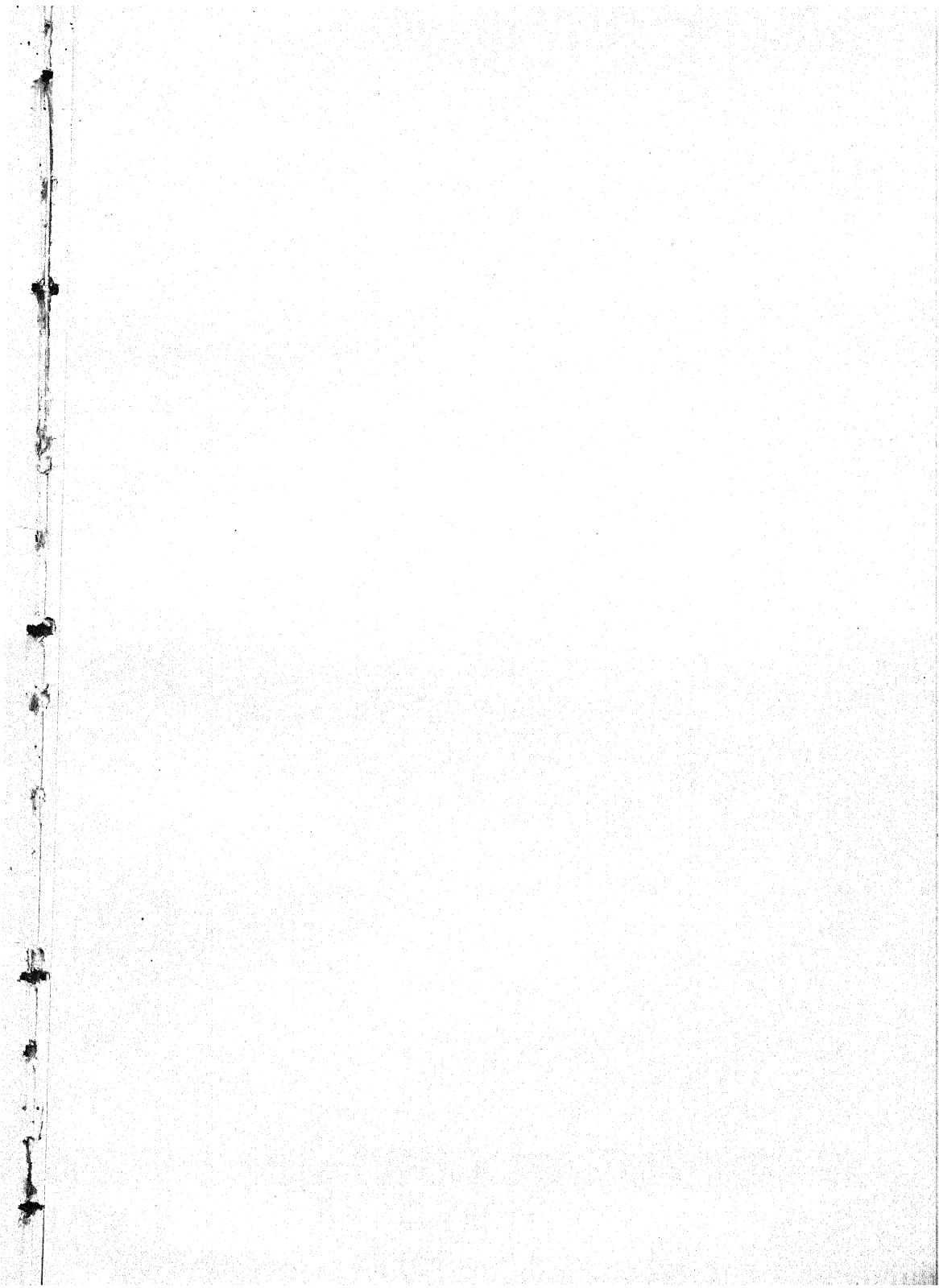
Fig. 1. Transverse section of part of the axis of Cone A of *E. maximum*. Note the torsion of one of the traces, the relatively numerous small tracheides, and the absence of carinal canals.  $\times$  circa 45.

Fig. 2. Transverse section of part of the axis of Cone C of *E. maximum*, showing the forking medullary strand, and between it and the normal bundles a small group of cells such as occurs below the end of medullary tracheides.  $\times$  circa 45.

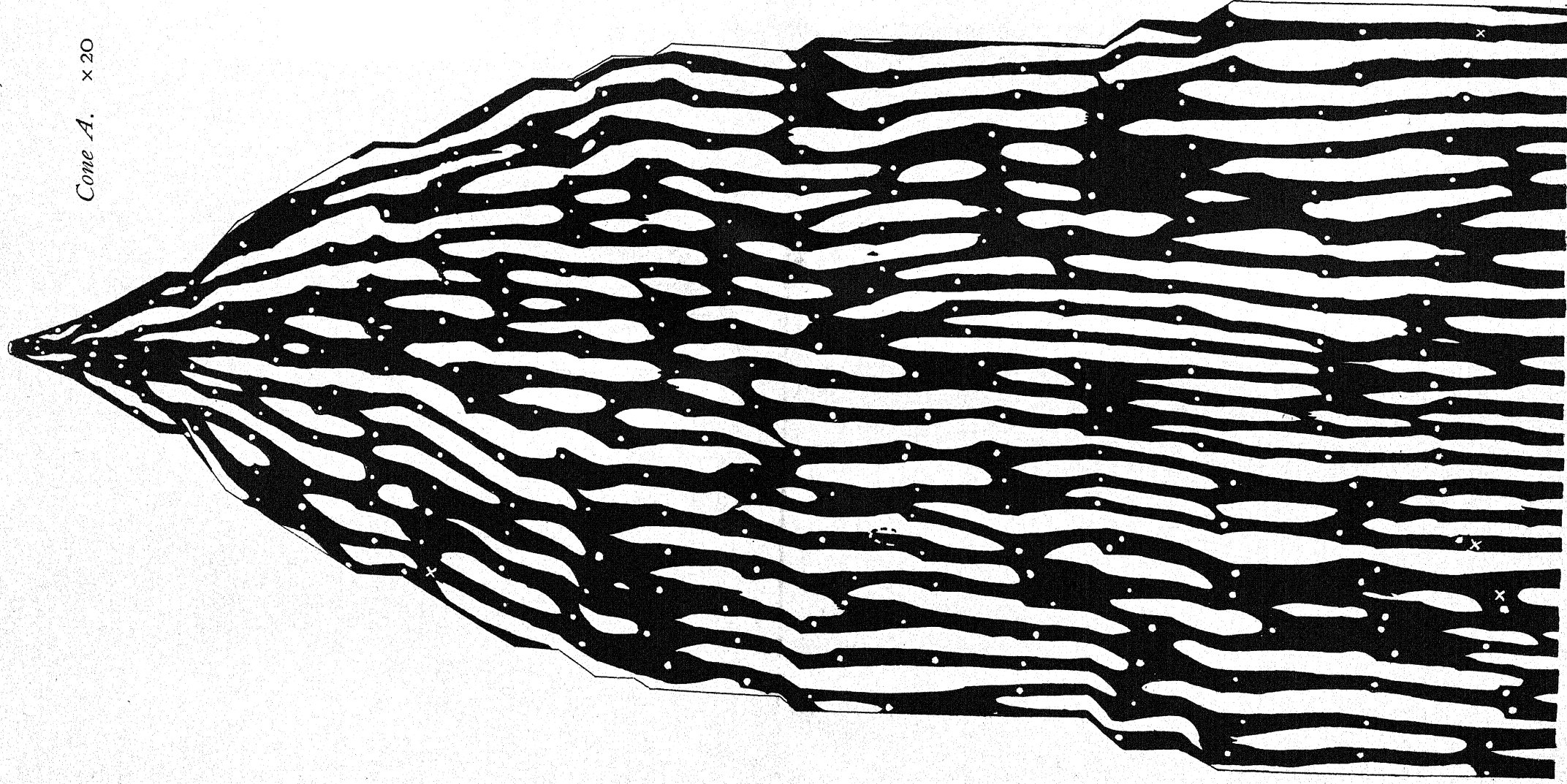
Fig. 3. Transverse section of part of the axis of Cone B of *E. maximum* at the level of the lowest whorl. Note the presence of some carinal canals and the small number of tracheides as compared with Fig. 1. Six traces are seen departing or passing through the cortex; those in the latter are mostly descending so sharply as to be cut nearly transversely.  $\times$  *circa* 45.

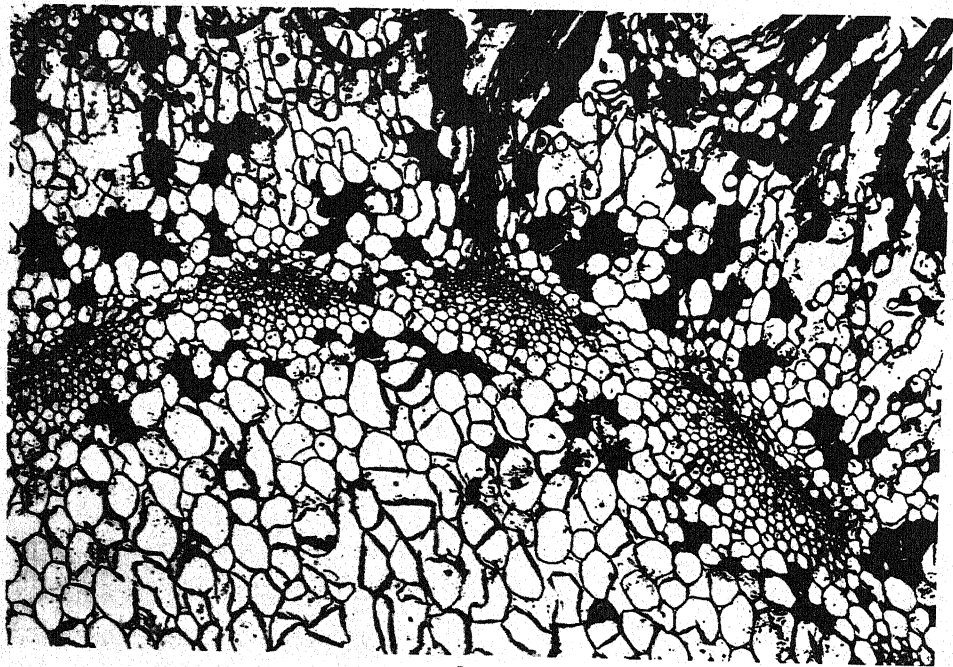
Fig. 4. Longitudinal section of part of the axis of Cone E of *E. maximum*, showing the annulus, the downward curvature (in this case not very great) of the trace of the lowest whorl, and the straight course of a trace of the third whorl (this section passes between the traces of the second whorl).  $\times$  *circa* 17.5.

Fig. 5. Longitudinal section of part of the axis of Cone E of *E. maximum*, showing the other side of the section shown in Fig. 4. Note the carinal canal on the inner side of the bundle on the internode above the annulus.  $\times$  *circa* 25.

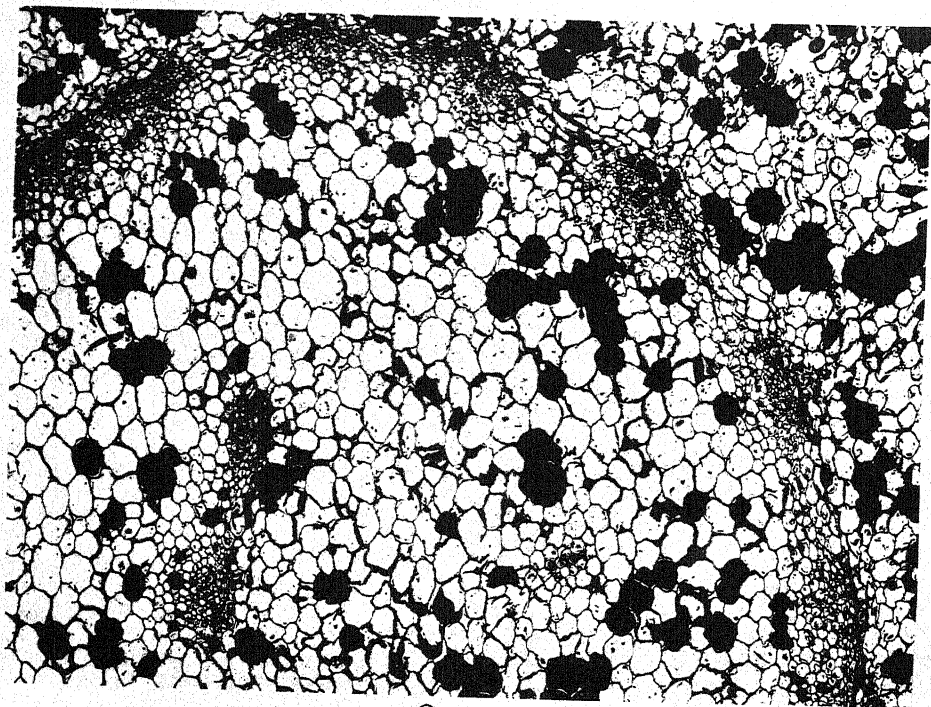


*Cone A.*     $\times 20$





1.

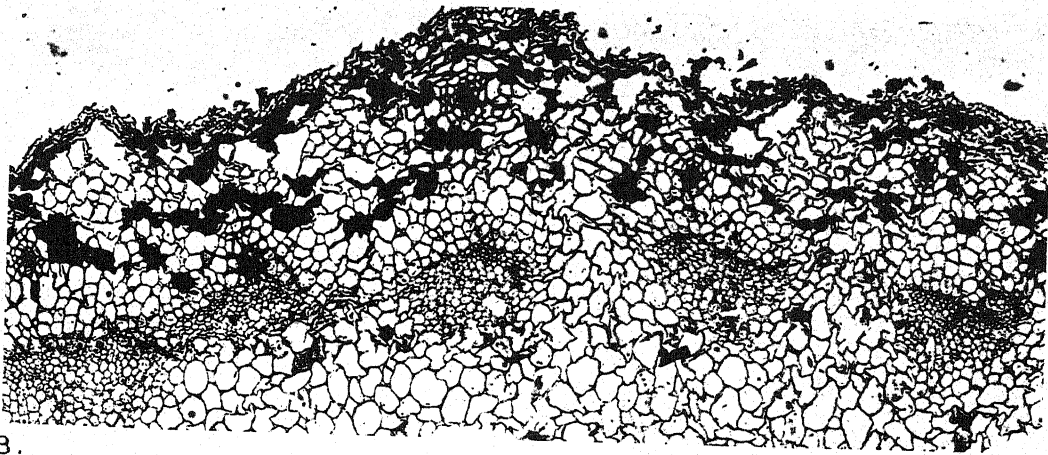


2.

Piddock phot.

BROWNE — EUISETUM.





3.



4.



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Huth coll.



# Hydrotropism in Roots of *Lupinus albus*.

BY

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## INTRODUCTION.

SINCE it was shown in a previous paper (16) that the phenomena commonly called thermotropic in roots were due largely to hydrotropism, it seemed advisable to investigate this tropism more in detail, and to determine to some extent the laws which govern it, and the limits within which it acts. The subject is of more particular interest because the question of the limitation of hydrotropic sensitivity to the root-tip has never been definitely settled since Darwin first suggested it. The work was done for the most part during the summer semester of 1913 at the University of Strassburg under the guidance of Professors Jost and Kniep, whose invaluable assistance I wish to acknowledge here. The work was finished at the Osborn Botanical Laboratory of Yale University at New Haven, Connecticut, under Professor Evans, to whom I am likewise indebted for much help.

## HISTORY.

The idea that roots enter the earth to seek moisture necessary for growth is one of those popular conceptions that are of venerable age because they are easily and naturally thought of. It was consequently offered as an explanation of those phenomena which we now call geotropic. Later, when botanists began to comprehend the nature of this latter reaction, the possibility of hydrotropism was questioned and experiments made which seemed to disprove its existence, for these two tropisms were then thought to be alternative. The study of hydrotropism has therefore experienced but slow development amid numerous set-backs, although its history has been relatively long.

As early as 1700 Dodart (7) tried to explain the directions taken by the root and shoot, as determined by the materials they need. He thought roots were made of fibres which shortened when acted on by the earth's moisture and lengthened when warmed by the sun's rays, while the stem

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fibres acted in exactly the reverse manner. Thus roots bent down and stems up.

Half a century later, Bonnet (1, p. 272) recorded an instance of hydrotropism when he admired the movement of roots which follow the undulations of a wet sponge.

Duhamel (9, vol. i, p. 86; vol. ii, pp. 137-45) was led to believe that large bodies of water might influence the direction assumed by the roots of near-by trees. To decide this, he made experiments. He placed an acorn upside down between two sponges, and again in pipes filled with earth and laid at various angles. As the radicle grew down and the plumule up in every case, Duhamel concluded that moisture had no effect on the directions assumed by the root and shoot.

Just a century after Dodart, Erasmus Darwin (5, p. 144) wrote: 'The plumula is stimulated by air into action and elongates itself where it is most excited, and the radicle is stimulated by moisture and elongates where it is most excited.'

The following year, Lefébure (21, p. 50) demonstrated nicely the existence of hydrotropism, although he did not realize it. A moist sponge was placed over some seeds in a nutshell, which was then inverted. The roots grew down and soon reached the air, whereupon they turned back into the sponge.

Knight (20), in an article read before the Royal Society ten years later, gave the first complete proof of hydrotropism in roots. *Vicia Faba* seeds were half embedded in the mould of inverted flower-pots. The radicles of the germinating seedlings had earth above and air below them. When the mould was kept moderately moist, the radicles extended horizontally along the under surface of the mould and sent side rootlets up into it. When the earth was supersaturated with moisture, the roots grew straight down into the air beneath. Knight held to the physical explanation of Dodart and E. Darwin, and emphatically denied the existence of anything resembling sensation or intellect in plants.

In endeavouring to test E. Darwin's statement, Keith (19) substantially repeated Duhamel's experiment. He used a kidney bean and a grain of wheat planted upside down in a glass tube. Although the radicles assumed a horizontal position after descending perpendicularly into the open air below the mould in the tube, Keith passed over this fact, because germination was then past. He merely wished to show that the primary cause of the descent of the radicle at germination was not sensitiveness to moisture.

In 1824 Dutrochet (10, pp. 59-60) made several experiments, including a repetition of Keith's, with *Phaseolus multiflorus*. He distinguished carefully between mass and moisture. In one experiment he fastened beans to the roof of an excavation where the earth above them was several metres thick. As the roots bent down, he concluded that mass of itself had no

influence. *Vicia Faba* seedlings were suspended near the surface of a wet sponge. The air was saturated and the roots did not bend, so Dutrochet decided that hydrotropism did not exist.

Five years later Johnson (17) was aroused likewise by E. Darwin's brief statement to test the influence of moisture on roots. An iron wire ring, on which was stretched some netting, was fixed in the mouth of an ale glass, filled with garden mould, and sown with mustard seeds. As the air at the bottom of the glass became moist from contact with the damp earth, the radicles grew straight down into it. A hoop was furnished with a thread-net bottom, filled with moist soil, and sown with mustard seed. As soon as the radicles penetrated the earth and the net, they turned to one side and crept along the under surface, perforating the net several times. He observed the same result when the seeds were placed in the pores of a sponge, fixed in the mouth of an inverted ale glass and cut off flush with the brim. Johnson concluded that roots were endowed with some force, different from and more powerful than that of gravitation, which compelled them to seek moisture.

Duchatre (8, p. 384) in 1856 gave a detailed criticism of the work of his predecessors. He experimented with *Chrysanthemum*, *Hydrangea*, and *Veronica*, and found that by keeping the air saturated and the soil dry, adventitious roots developed around the base of the stem and showed no tendency to descend to earth. Several roots came out of the earth and lay on the surface or even ascended into the moist air.

Van Tieghem (32, p. 325) in 1869 explained the direction taken by germinating pollen tubes as due to their sensitiveness to moisture. 'The pollen tubes finding moisture on only one side, bend in that direction and penetrate the stigma.' The statement was copied by Capus (2, p. 282) nine years later.

Ciesielski (3, p. 25, fig. 5) in 1871 observed that a *Zea Mays* root laid horizontally on a water surface, which wets the under side only, bends upward in the usual bending zone. The same thing resulted over a wet solid surface, and could be obtained with oat and wheat seedlings as well. Ciesielski considered this upward bending to be a phenomenon of growth which illustrated a peculiar theory that he was advancing.

Sachs (27) reinvestigated the entire field of hydrotropism and placed it on a firm and substantial basis. He experimented with roots of *Pisum*, *Phaseolus*, *Vicia*, *Zea*, *Helianthus*, *Tropaeolum*, and *Ipomaea*, and always took the precaution of working in a dark room. He found a hanging sieve with zinc sides and a cloth bottom the most satisfactory means of obtaining hydrotropic bending. It was filled with moist sawdust, sown with seed, and hung at an angle of 45°. The results agreed with those of Knight and Johnson. By hanging the sieve at an angle the projecting root was subjected to a difference of moisture on the two sides, and so bent to that part

of the sieve-bottom nearest it. Sachs mentions several less satisfactory substitutes for the sieve, such as peat bricks, plaster of Paris plates, sponges, or bags of moist earth. His attempt to explain hydrotropism as caused by thermotropism failed (see 16, pp. 135-6), so he was forced to conclude that the bending was produced directly by the moisture difference. Duchatre's and Ciesielski's results he considered to be produced by alterations in the turgidity of the roots. Shoots were found to be hydrotropically insensitive, for they grew straight out of the sawdust in the tilted sieves.

Van Tieghem (33) in 1876 observed the formation of arcades by the stolons which give rise to sporangiophores in *Absidia*, and attributed their bending to 'somatropism' or the attraction of mass. He found stolons of *Circinella*, *Mortierella*, *Mucor*, *Pilobolus*, and *Phycomyces* all positively somatotropic. Three years later Sachs (28, p. 224) showed Van Tieghem's somatropism to be untenable. He suggested that the vegetative hyphae of *Phycomyces* and *Mucor* were positively, and their sporangiophores negatively, hydrotropic.

Charles Darwin (4, pp. 180-6) held that the hydrotropic sensitivity of radicles resided in their tips. He used Sachs' method for obtaining the bending with seedlings of *Phaseolus*, *Vicia*, *Avena*, and *Triticum*. To check the bending, from one to two millimetres of the root-tip were coated with a mixture of olive oil and lamp-black. Of fifty-nine roots so treated, twenty-nine remained straight. One millimetre of the tip was cauterized with silver nitrate in other experiments. Of twenty cauterized roots, eleven remained straight after twenty-four hours. Darwin wrote that 'a greater number of experiments than those which were actually tried would have been necessary had it not been clearly established that the tip of the radicle is the part which is sensitive to various other irritants'.

Wiesner (35, pp. 130-4) objected to Darwin's method, on the ground that the roots were in an abnormal condition. His own experiments with decapitated roots led him to the conclusion that hydrotropic sensitivity was not confined to the root-tip. The term 'hydrotropism' appears here for the first time in the literature.

Detlefsen (6, pp. 646-7) also criticized Darwin's method and results. An experiment with six decapitated pea seedlings, four of which reacted, convinced him that the entire growing region of the root perceived the stimulus.

Mer (22) offered a 'more natural' explanation for hydrotropism. After detailed observations on germinating lentil seedlings, he decided that roots were pulled to one side by the attachment of root-hairs, and that they possessed no special hydrotropic faculty.

The same year Wortmann (36, pp. 368-74) showed that *Phycomyces nitens* sporangiophores were negatively hydrotropic. A sporangiophore projecting through a hole in a glass plate was found to bend away from

a wet piece of cardboard, but to grow straight by a dry piece. The mycelium was not found to be hydrotropic.

In 1883 Molisch (24) published a comprehensive work on hydrotropism. He constructed the funnel apparatus for obtaining hydrotropic bendings. The projecting root-tips of seedlings placed on the top of the solid plaster of Paris funnel bend first down and then inward to the moist side of the funnel. Molisch demonstrated that hydrotropic bending resulted from unequal growth of the opposite sides of the root. Roots of *Zea* and *Pisum* seedlings were marked with ink every millimetre, and the region of hydrotropic bending was thereby found to be the growing region. Reactions were inhibited below the minimum temperature for growth. Hydrotropically bent roots were plasmolysed, and remained bent, showing that the reaction was not a phenomenon of turgidity. He observed that disturbances of the turgidity caused by a psychrometrical difference frequently bent the root away from the source of moisture. Molisch agreed with Darwin that the root-tip alone received the hydrotropic stimulus, and gave positive proof that 1.5 mm. of the root-tip was sensitive. The part of the root above the tip was wrapped in wet tissue-paper, and the wrapping was shoved down as the root grew, so that more than 1.5 mm. was never exposed to the moisture difference. Molisch found side rootlets especially sensitive to hydrotropism. The rhizoids of *Marchantia polymorpha*, *Lunularia*, and *Fegatella* were found to be positively hydrotropic; the sporangiophores of *Mucor* and *Coprinus* and the hypocotyl of *Linum usitatissimum* were shown to be negatively hydrotropic.

Elfving (12) in 1890 observed that sporangiophores of *Phycomyces nitens* bent towards pieces of iron and various other substances, and described the process as physiological action at a distance. Two years later Errera (14) pointed out that all of these substances were hygroscopic, so that the real cause of the bending was negative hydrotropism. He found that roots bent away from iron even in a saturated atmosphere, 'which shows that hydrotropism is not due, as generally admitted, to differences in the hygrometric state of the air. Hydrotropism itself is the bending of a plant towards a point, not where it will find a minimum or maximum of moisture, but where it will, within certain limits, transpire most or least.' Elfving (13) replied in 1893 that potash, although hygroscopic, did not cause the sporangiophores to bend, and that various other inactive substances were rendered active by exposure to sunlight or to heat. Errera (15) wrote in 1905 that although sure of the exactness of his former experiments, Elfving's more recent experiments should be repeated in the light of our recent knowledge concerning the radiation of metals.

In 1894 Miyoshi (23) demonstrated that pollen tubes of *Epilobium angustifolium*, *Oenothera biennis*, *Oe. fruticosa*, *Digitalis grandiflora*, and *D. purpurea* were positively hydrotropic.

The same year Rothert (26) published a critical study of the literature on the function of the root-tip. He pointed out that Darwin's theory was as yet unproved. The converse of Molisch's experiment would determine the point, however. This had been done in Pfeffer's laboratory, but as no details were given definite conclusions could not be drawn in view of numerous possible sources of error.

In 1901 Steyer (31) thoroughly investigated the hydrotropism of *Phycomyces nitens*. He found that the sporangiophores were both negatively and positively hydrotropic according to the percentage of relative moisture. Steyer was unable to find any foundation for Elfving's 'physiological action at a distance'. Older sporangiophores were found to be more sensitive than younger ones, and exposure to light lessened their sensitivity.

Voechting (34, pp. 98-102) in 1902 experimented with potato sprouts and decided that they were hydrotropic. The following year Singer (29) showed that Voechting's results were probably due to impure laboratory air, and that potato sprouts were not hydrotropic.

Sperlich (30) in 1908 observed that the stolon of *Nephrolepis* was positively hydrotropic, thus enabling it to reach a moist substratum in spite of the absence of geotropism.

The last contribution to the literature on hydrotropism was made by Jost and Stoppel (18, p. 210) in 1912, and dealt with the limitation of hydrotropic sensitivity to the root-tip. Of eighteen decapitated *Lupinus albus* roots arranged a few millimetres from a wet filter-paper, thirteen bent to the paper. This indicates that although the strongest hydrotropic sensitivity resides in the tip, it is not confined to it.

Up to the present time hydrotropism has been found in the sporangiophores of various fungi, in the rhizoids of hepatics, in roots and pollen tubes, and in rare cases in the hypocotyl of the spermatophytes.

#### EXPERIMENTAL PART.

1. *Method.* The simplest contrivance for obtaining hydrotropic bending in roots was constructed in the following manner: A glass plate covered on both sides with wet filter-paper was inserted in a small rectangular glass jar parallel with the longer sides. Roots were fastened to pins which pierced strips of cork at regular intervals. Two strips were laid across the top of the jar on either side of the glass plate and parallel with it. The roots thus brought into the jar were exposed on one side to the moisture of the filter-paper, and their distance from the paper could readily be controlled. The bottom of the jar was covered with water to keep the filter-paper wet and to prevent the roots from drying out. The jar was left open that the air in it might not become saturated and so prevent

a psychrometrical difference about the roots. For relatively rough work this method was found very effective.

In order to make careful measurements a more complicated apparatus was constructed. A zinc vessel 10 cm. broad, 20 cm. high, and 30 cm. long was used. The sides were of glass, and were covered with flaps of black paper to shut out the light in case the experiment was not made in a dark room. The box was supplied with a tightly fitting cover. One end of the vessel was covered on the inside with filter-paper. On the floor 2 cm. from this end, a piece of glass 2 cm. high and 10 cm. long was fixed upright and the connexions with the sides and bottom were made water-tight. This formed a reservoir, which collected water at the base of the filter-paper. Two short tubes penetrated the zinc wall behind the filter-paper, one near the top, which served as an inlet, and one near the bottom, which acted as an overflow from the reservoir. The former could be connected with the faucet and running water supplied to the filter-paper from above, which flowed down it to the reservoir at its base and made its exit through the lower opening. In the earlier experiments the water could not safely be left running, so a constant supply of water was obtained by other means. A bent glass tube, the lower end of which was drawn out to a capillary, supplied water from a beaker. By breaking off the capillary at the proper point, the amount of water supplied could be perfectly regulated. In this way a constant source of moisture was produced at one end of the closed compartment. The air within never became completely saturated, although the cover was kept on. This ensured a definite decrease in the percentage of relative moisture from one end to the other. This decrease could be augmented or diminished by placing at the drier end of the compartment a tray filled with sulphuric acid or water respectively. Mouldings extended the length of the two sides about 2 cm. from the top. These supported strips of cork to which seedlings had been pinned, which could thus be placed at any desired distance from the filter-paper. Evaporation from the cotyledons was found to have considerable influence on the prevailing moisture conditions, so a method was devised to obviate it. Zinc trays were constructed 9.5 cm. long, 2 cm. wide, and 3 cm. deep. These had covers, and were arranged to hang from the mouldings on the opposite sides of the compartment. Five holes were made in the bottom of the trays, through which the roots of the seedlings were inserted or allowed to grow. The cotyledons were packed with moist sawdust, with which the trays were completely filled. The bottom of the tray was coated on the outside with paraffin that any hygroscopic action of the zinc might be avoided. In this way only the *roots* which were to be acted on hydrotropically came under the artificial conditions of the zinc compartment. The flank sides of the roots were turned towards the filter-paper.

Specially constructed hygrometers were used to measure the percentages of relative moisture within the zinc compartment. A grain of *Stipa pennata* was inserted in a small cork and the awn was cut off at an appropriate distance, leaving from 1 cm. to 2 cm. of it projecting vertically from the cork. A glass capillary about 2 cm. long was fixed with sealing-wax to the cut end of the awn so that it extended horizontally. This served as an indicator, turning as the hygroscopic awn twisted tighter. Markings were made on a circular piece of cardboard fastened to the top of the piece of cork. This hygrometer was calibrated by placing it in a small closed compartment with a relatively large amount of a definite concentration of sulphuric acid. The percentage of relative moisture was determined by the vapour pressure of the sulphuric acid.

TABLE I.<sup>1</sup>

| Percentage<br>of<br>$H_2SO_4$ . | Vapour<br>pressure<br>at 20° C. | Relative<br>percentage of<br>moisture<br>approximately. |
|---------------------------------|---------------------------------|---|
| 57.65                           | 3.728                           | 21  |
| 52.13                           | 5.792                           | 33  |
| 43.75                           | 8.494                           | 49  |
| 37.69                           | 10.831                          | 62  |
| 33.10                           | 12.317                          | 71  |
| 24.26                           | 14.482                          | 83  |
| 0.00                            | 17.363                          | 100   |

By using several different sulphuric acid solutions, the hygrometers could be rendered accurate within 5 per cent., but they had to be recalibrated every three weeks. The instruments were carefully compared with one another and were found to vary from one another within 2 per cent. The measurements of psychrometric differences would then be accurate within 2 or 3 per cent., which is sufficient for the present purposes.

A hygrometer was placed at either end on the floor of the compartment, their centres 25 cm. apart. Under ordinary circumstances a difference of 8 per cent. was registered.

The seeds of *Lupinus albus* were soaked twenty-four hours in water and allowed to germinate in sawdust until the roots had reached a suitable length. To be sure there are other seedlings which react better to hydrotropism than lupins, as for example corn seedlings. But great difficulty was experienced in obtaining straight roots of corn that would suit for experimentation. In this and other respects lupin seedlings were found to be most satisfactory.

The experiments were conducted for the most part at a temperature of 20° C.

<sup>1</sup> Compiled from Landolt and Börnstein: Physikalisch-chemische Tabellen, pp. 360, 426. Vierte Auflage. Berlin, 1912.

2. *Limits of the reaction.* The first point that was investigated was within what limits of relative moisture hydrotropic reactions take place in roots. The upper limit is evidently saturation. The lower limit was found to be determined by the inability of roots to grow in air that is too dry. As no results have been observed in the literature which determined the amount of water vapour requisite for growth, the following experiments were made. A number of Erlenmeyer flasks were fitted with corks, and a hole was bored through each cork. Seedlings about 3 cm. long were selected, and the roots inserted through the hole. Only one root was placed in each flask, as several were found to alter materially the moisture-content of the enclosed air and to have a strong reciprocal influence. The vapour pressure within the flasks was regulated by sulphuric acid solutions. The roots were marked with ink and measured before and after the experiment, to determine the amount of growth during the twenty-four hours. The percentages of relative moisture were calculated from the vapour pressures.

TABLE II.

| Percentage<br>of $H_2SO_4$<br>in water. | Vapour<br>pressure. | Relative<br>percentage of<br>moisture. | No. of roots<br>experimented<br>with. | Average<br>growth<br>per hour. |
|---|---------------------|--|---------------------------------------|--------------------------------|
| 0.00                                    | 17.0                | 100                                    | 30                                    | 0.75 mm.                       |
| 7.0                                     | 16.5                | 95                                     | 35                                    | 0.52 mm.                       |
| 14.0                                    | 15.6                | 90                                     | 30                                    | 0.22 mm.                       |
| 21.0                                    | 14.7                | 85                                     | 30                                    | 0.15 mm.                       |
| 27.0                                    | 13.9                | 80                                     | 25                                    | 0.00 mm.                       |

In these experiments the cotyledons were left exposed to the laboratory air. If these are kept moist by wrapping them in wet cotton, the average growth per hour is greater, but the roots within the flasks stop growth and dry up if the relative moisture is reduced to 80 per cent. Seedlings grown in a compartment with saturated air may lengthen as much as 1.13 mm. per hour.

Reaction to hydrotropic stimuli ceases, however, slightly above 80 per cent. relative moisture; evaporation then becomes so great and growth so slow that loss of turgidity is apt to come into play and to cover the effects of any hydrotropic stimuli, if such are perceived. Roots subjected to a psychrometrical difference at such percentages often bend away from the source of moisture. This is not to be confused with negative hydrotropism, which is not known to exist in roots (18).

3. *Intensity of the reaction.* The effects of varying the intensity of the hydrotropic stimulus were next investigated. The zinc compartment and the hygrometers made from *Stipa pennata* grains were used to make the measurements. A noticeable difference in the rate of growth of roots situated at various distances from the filter-paper became evident before hydrotropic bending was observed. Each tray held five roots.



## EXPERIMENT I.

| No. of tray. | Distance from filter-paper. | Average growth. | Growth per hour. |
|--------------|-----------------------------|-----------------|------------------|
| I            | 5 cm.                       | 16.8 mm.        | 0.93 mm.         |
| II           | 10 cm.                      | 11.0 mm.        | 0.61 mm.         |
| III          | 15 cm.                      | 8.4 mm.         | 0.46 mm.         |
| IV           | 20 cm.                      | 7.2 mm.         | 0.40 mm.         |

This experiment covered eighteen hours. One hygrometer 3 cm. distance from the filter-paper measured 97 per cent., and another 28 cm. distant measured 90 per cent. relative moisture. Three roots in I, two each in II and III, and one in IV bent towards the filter-paper. The reaction was observed in I and II at the end of seven hours, and in III and IV half an hour later.

## EXPERIMENT II.

| No. of tray. | Distance from filter-paper. | Average growth. | Growth per hour. |
|--------------|-----------------------------|-----------------|------------------|
| I            | 5 cm.                       | 19.4 mm.        | 0.97 mm.         |
| II           | 10 cm.                      | 17.2 mm.        | 0.86 mm.         |
| III          | 15 cm.                      | 11.6 mm.        | 0.58 mm.         |
| IV           | 20 cm.                      | 6.2 mm.         | 0.31 mm.         |

The experiment lasted twenty hours. The hygrometers measured 87 and 98 per cent. respectively. After six hours three in I began to bend, and an hour later four in II, and two each in III and IV, bent towards the filter-paper. The remaining roots grew straight.

## EXPERIMENT III.

| No. of tray. | Distance from filter-paper. | Average growth. | Growth per hour. |
|--------------|-----------------------------|-----------------|------------------|
| I            | 5 cm.                       | 18.2 mm.        | 0.76 mm.         |
| II           | 10 cm.                      | 11.0 mm.        | 0.46 mm.         |
| III          | 15 cm.                      | 8.6 mm.         | 0.25 mm.         |
| IV           | 20 cm.                      | 6.0 mm.         | 0.25 mm.         |

This experiment lasted twenty-four hours. The hygrometers measured 88 and 96 per cent. respectively. After six and a half hours three roots in I and two in II and IV bent positively towards the source of moisture.

## EXPERIMENT IV.

| No. of tray. | Distance from filter-paper. | Average growth. | Growth per hour. |
|--------------|-----------------------------|-----------------|------------------|
| I            | 5 cm.                       | 17.6 mm.        | 0.80 mm.         |
| II           | 10 cm.                      | 9.8 mm.         | 0.45 mm.         |
| III          | 15 cm.                      | 6.2 mm.         | 0.29 mm.         |
| IV           | 20 cm.                      | 3.6 mm.         | 0.16 mm.         |

The experiment lasted twenty-two hours. A tray with sulphuric acid (90 per cent.) was placed in the end opposite the filter-paper. The hygrometers measured 83 and 95 per cent. respectively. One root each in I, III, and IV bent positively after seven and a half hours. One root each in III and IV was curved negatively.

EXPERIMENT V.

| No. of tray. | Distance from filter-paper. | Average growth. | Growth per hour. |
|--------------|-----------------------------|-----------------|------------------|
| I            | 5 cm.                       | 18.2 mm.        | 0.90 mm.         |
| II           | 10 cm.                      | 16.0 mm.        | 0.80 mm.         |
| III          | 15 cm.                      | 13.3 mm.        | 0.67 mm.         |
| IV           | 20 cm.                      | 10.8 mm.        | 0.54 mm.         |

The experiment was continued twenty hours. A tray with dilute sulphuric acid (10 per cent.) was placed in the dry end of the compartment. The hygrometers measured 88 and 93 per cent. respectively. Only one root (in III) bent towards the filter-paper. The others remained straight.

This last experiment showed that if the hygrometers measured a difference of only 5 per cent., hydrotropic reaction was very nearly eliminated. If this difference was less, no reactions occurred. The hygrometers were 25 cm. apart, so it may be said that a fall of at least 0.2 per cent. in 1 cm. is necessary to induce hydrotropic reaction. This represents approximately the minimum intensity of the stimulus. If the other extreme is obtained, the roots are affected by changes in turgidity and bend away from the source of moisture as in Experiment IV. The exact point where this occurs could not be determined; the hygrometers were too inaccurate. The phenomenon is familiar, however, and was mentioned by Molisch (24). The optimum reaction was found to be obtained when a psychrometric difference of 0.4 per cent. for every centimetre was measured. This means a difference of but 0.04 per cent. between the opposite sides of a root 1 mm. thick.

Repeated experiments with the zinc apparatus showed that the roots in tray I bent normally but 20° to 30° from the vertical, while those in tray V bent from 50° to 60°. To explain this difference a detailed study of the moisture content of the air in the compartment was necessitated. When the compartment was empty the fall in moisture content was found to be more or less uniform. The presence of the roots, however, had a significant influence on this, so that experiments having any bearing on the question had to be made with the roots in their accustomed positions within the zinc box. Water must evaporate from the surface of a root suspended in moist air, unless the air is saturated. Consequently each root acts as a source of moisture and tends to increase the moisture content of the air about it. The same was observed in the experiments made to determine the minimum relative moisture. As a result of this increase, the fall in the relative moisture of the moister end of the zinc compartment, which contained more of the roots during these experiments, was diminished. The data for the following table were collected from ten experiments in which the hygrometers at the two ends of the apparatus measured approximately 98 per cent. and 90 per cent. The relative moisture was then measured by hygrometers at intervals of 5 cm. throughout the entire length of the

compartment and the average for each position calculated. In every case the experiments were made with the roots in their customary positions.

TABLE III.

| <i>Distance from the filter-paper.</i> | <i>Average percentage relative moisture.</i> | <i>Distance from the filter-paper.</i> | <i>Average percentage relative moisture.</i> |
|--|--|--|--|
| 3 cm.                                  | 98.2   | 18 cm.                                 | 94.9   |
| 8 cm.                                  | 97.0   | 23 cm.                                 | 92.7   |
| 13 cm.                                 | 96.1   | 28 cm.                                 | 90.1   |

This shows that the fall in moisture is greater at the dry end of the apparatus. The psychrometric difference would consequently be greater there than at the moister end of the compartment, which explains the greater intensity of reaction observed.

The results of numerous experiments similar to those described in detail has been summed up in the following table. Only those roots were considered that reacted, and, moreover, only those in trays I, II, and III, in order that the results might be comparable. The intensity of the stimulus was calculated from the differences measured by the hygrometers at 20° C. and is expressed in percentages of relative moisture per centimetre. The bending was positive unless otherwise stated, and the averages were reduced to round numbers.

TABLE IV.

| <i>Intensity of stimulus.</i> | <i>No. of roots observed.</i> | <i>Average angle of bending.</i> |
|-------------------------------|-------------------------------|----------------------------------|
| 0.1                           | 45                            | 0°                               |
| 0.2                           | 32                            | 0°                               |
| 0.3                           | 86                            | 30°                              |
| 0.4                           | 60                            | 60°                              |
| 0.5                           | 20                            | 10°                              |
| 0.6 and above                 | 34                            | negative                         |

The simpler apparatus for obtaining hydrotropic bending was rotated horizontally about its vertical axis on a clinostat. The action of geotropism was thus eliminated, while the hydrotropic stimulus was in no way affected. Of twenty roots so rotated, seventeen reacted and with the same intensity as the controls. This showed that geotropism was not a factor in determining the intensity of hydrotropic bending.

4. *Reaction time.* Under optimum conditions, i.e. when the hygroscopic difference near the root is equivalent to a fall of 2 per cent. in 5 cm. and when the absolute amount of moisture is above 90 per cent., roots require six hours to start a hydrotropic reaction. The bending proceeds for one to two hours, whereupon a reaction sets in, and the root-tip regains a vertical position. Under favourable circumstances the entire reaction, from the time when the psychrometric difference is established until the root-tip regains its vertical position, may be completed in eight hours.

The average time that elapsed before a reaction was visible was seven hours. In case of unfavourable conditions, due to a low stimulus intensity, or to slow growth, ten hours elapsed before bending could be detected.

Roots were exposed to the influence of a moist filter-paper for periods varying from one minute to five hours, in the hope of determining a presentation period such as exists for heliotropism and geotropism. The results were negative as no reaction was obtained unless the roots were exposed more than five hours.

5. *Localization of sensitivity.* The greatest point of discussion in regard to hydrotropism has been whether the sensitivity is confined to the root-tip or no. Darwin, Molisch, and Pfeffer were of this opinion, while Wiesner, Detlefsen, and Jost gave evidence against the view. By the root-tip is meant one and one-half to two millimetres of the end of the root. Several methods were tried and found unsatisfactory before one was used which decided the question.

Molisch's experiment (24) was repeated, in which roots wrapped up to the tip in wet tissue-paper were exposed to a hydrotropic stimulus. Good results were obtained, although the roots reacted somewhat more slowly than under optimum conditions.

A. Then the converse experiment was made, which Pfeffer had carried out in his laboratory (25). The root-tips alone were covered and the roots then exposed to a psychrometrical difference. Tissue-paper and tin-foil were both used, but the only cases in which bending occurred seemed due rather to pressure of the cap on the root-tip, for the roots bent in all directions.

B. The method used by Jost (18) was next tried, and 1.5 to 2 mm. of the tip were carefully removed with a razor. Of fifty-four roots of *Lupinus albus* which were decapitated, 20 bent towards the filter-paper, 11 bent away, and 23 remained straight or bent to one side. The greatest care was used to cut the roots squarely, and the experiment was not begun until some little efficiency had been attained. To the last, nevertheless, a large percentage (20-25 per cent.) of the controls bent in reaction to a wound stimulus. The reaction took place sometimes in two to three hours, and so had clearly no relation to hydrotropism. The results of the experiment were characterized by the greatest irregularity, so that this method was also abandoned.

C. The tips of roots were killed by immersion for two minutes in boiling water, but the rate of growth was seriously diminished, so that no results could be obtained.

D. A rectangular glass vessel was half filled with water. Roots suspended from two parallel cork strips, resting on the rims of the vessel, were immersed for a distance of 1 to 2 mm. from the apex. Between the two rows of roots, a paraffin trough was fixed at the water

level, and was filled with a few drops of concentrated sulphuric acid. This produced a psychrometrical difference on the two sides of the roots, which, however, could not be measured as the hygrometers were too large. The whole apparatus was enclosed in a covered jar to obviate disturbing air-currents and placed in a dark cupboard. As the roots grew into the water they were pulled out of it by means of the pins to which they were fastened, and in this way never more than 2 mm. were immersed. Of 94 roots treated in this way, 50 bent away from the sulphuric acid, 8 towards it, and 36 remained straight or bent to one side. Although these results indicate that hydrotropic reactions may occur if the root-tips do not receive a direct stimulus, they nevertheless were considered unconvincing.

An attempt was made to grow the roots in water first, to accustom them to that medium before the experiment just described was begun, but roots thus grown were found to be very insensitive to hydrotropic stimuli, and the greatest difficulty was met with in obtaining straight roots.

E. The last experiment was repeated, with the difference that paraffin oil was substituted for water in the bottom of the glass vessel. The floating paraffin tray was fixed about 2 mm. from the two rows of immersed root-tips. This apparatus was enclosed in a jar, the air of which was kept as nearly as possible saturated. Of 117 roots, 81 bent away from the sulphuric acid, 12 towards it, and the remaining 24 stayed more or less straight. The reactions required eight to nine hours. These results were accepted as decisive.

In discussing the perceptive region of roots, Rothert (26) made four categories to include all possibilities:

1. Only a relatively short tip may be sensitive.
2. The whole end from the growing region to the apex may be sensitive, the tip however to a greater degree.
3. The whole end from the growing region to the apex may be equally sensitive throughout.
4. The whole end except the tip may be sensitive.

The second category has been shown to apply to hydrotropism. Molisch (24) demonstrated that the tip was highly sensitive, and the previous experiment shows that the region above the tip is also capable of receiving hydrotropic stimuli, but since more time was required for the reaction this region is probably less sensitive. The cells appear to lose their hydrotropism in proportion as they lose their embryonic qualities. It seems possible that some connexion may exist here, for differentiation of the root-cells would naturally render them ineffective for other purposes than those for which they become modified.

6. *Nature of hydrotropism.* Darwin (4) considered hydrotropism to be precisely the opposite of traumatropism, for in the one case the roots bend

towards the source of moisture, and in the other away from the side wounded. Molisch (24) considered that the roots bent away from the dryness, rather than towards the moisture, and so came to the conclusion that hydrotropism was merely a special kind of traumatropism. That this conception is fallacious is evident by careful examination of the data given. Hydrotropic reactions are obtained best between 90 and 100 per cent. of relative moisture; they cease slightly above 80 per cent.; while roots become noticeably injured from dryness below 80 per cent. If roots are injured, they bend away from the source of moisture, because the turgidity of the exposed side is lost. It is therefore not possible to maintain that hydrotropism is due to reaction from injury. Molisch based his idea on experiments made in an atmosphere of 72 per cent. relative moisture. Without doubt many of the roots sustained injuries.

To gain a correct conception of the nature of hydrotropic reaction in roots, it will be necessary to analyse the factors involved. First will be considered the mechanical effects resulting from exposing a root to a moisture difference in the air. As the root contains more water than the surrounding atmosphere, evaporation will result. This loss of water will produce increased osmotic pressure and decreased turgidity of the root-cells. The length of the cell decreases with the lessening of the turgidity, just as it does before plasmolysis. Since evaporation is greater from one side than from the other, the drier side will shorten more, and the root will bend away from the source of moisture. This reaction was obtained experimentally by pressing seedlings against the vertical surface of an agar block. The air contained 50 per cent. relative moisture. In fifteen minutes 11 out of 20 root-tips had bent away from the block, and in thirty minutes more all but two had reacted. The controls placed in a saturated atmosphere remained straight. The negative bending described in Experiment IV was of this nature.

But it is still a question if this mechanical tendency to bend negatively is present under those circumstances which produce positive hydrotropic reaction. In order to eliminate the vital factor, which will be discussed presently, roots were anaesthetized by exposing them twenty minutes over 3 per cent. ether water. The roots were then exposed to a hydrotropic stimulus before a moist filter-paper. Of the 25 roots exposed, 12 bent away from the paper within an hour, and 10 more within four hours. Sixteen out of 20 controls reacted hydrotropically. The exposure to ether water rendered the roots hydrotropically insensitive, without stopping their growth. This experiment shows that even those minimal differences of moisture content which induce hydrotropic bending produce a tendency for the root to bend negatively. This is readily explained by considering the mechanical tensions normally present behind the root-tips. If a thin radial section of a root, taken 5 to 8 mm. from the tip, is

divided longitudinally down the middle, the two halves become concave on the inside and bend away from one another, when a drop of water is placed on them. This experiment shows that a region of negative tension is present in the centre of the growing root, and that it is surrounded by a zone of positive tension. In other words, the young root is in a condition of unstable equilibrium. When the positive tension is reduced on one side by evaporation, the root bends.

It has been shown that if roots reacted solely to mechanical forces resulting from a moisture difference, they would bend negatively. That they bent positively can only be explained by assuming the presence of a vital factor, which must be powerful enough to overcome the mechanical factor. The moisture difference produces a difference in the osmotic pressure of the cells on the opposite sides of the root. It is natural to conclude that this difference may cause the bending. If an increased osmotic pressure acts as a stimulus to growth, the explanation of hydrotropism is simple. Moreover, it is known that the growth of certain sea Algae is inhibited by transferring them from salt to fresh water.<sup>1</sup> Here the decrease in osmotic pressure acts as a stimulus and retards or stops growth. The data in Table II would indicate that this is not the case in roots, but as the roots in these experiments had no means of obtaining water, which is essential for growth, no conclusions may be drawn from them in regard to this question.

In the following experiments roots were exposed to air of various degrees of dryness, in order to produce a stimulus by increasing the osmotic pressure through evaporation. The roots were then placed in moist sawdust, and the amount of growth compared with controls. The amount of relative moisture in the air was determined by using sulphuric acid solutions as in Table II.

*Experiment I.* Forty-seven roots were exposed for four hours in an atmosphere of 85 per cent. relative moisture. After five hours in moist sawdust, the amount of growth was measured and found to average 3 mm. Forty-eight controls were placed for four hours in a saturated atmosphere. The average growth for five hours in moist sawdust was 6.5 mm.

*Experiment II.* Ninety-six roots were exposed for  $4\frac{1}{2}$  hours to 90 per cent. relative moisture. They averaged for four hours' growth in sawdust 3.6 mm. Eighty roots, after being the same length of time in a saturated atmosphere, averaged 3.7 mm. for four hours' growth in moist sawdust. Eighty-five roots that had been germinated in the sawdust were measured and replaced. They averaged 3.3 mm. for four hours' growth.

*Experiment III.* Forty-eight roots were placed for one hour in a chamber having a relative moisture of 90 per cent. Their average growth for the following five hours measured 5.3 mm. Forty-eight controls exposed

<sup>1</sup> Jost's Pflanzen-Physiologie, p. 348. Dritte Auflage. Jena, 1914.

for one hour to a saturated atmosphere averaged 5.25 mm. for five hours in moist sawdust.

These results show that an increase in the osmotic pressure does not stimulate growth directly. Although the vital factor may not be explained so simply, it may yet be stimulated to action by the difference in osmotic pressure. As long as one side of the root has a higher osmotic pressure, there must be a flow of water across the root brought about by diffusion from cell to cell. In each cell there would exist a difference in the osmotic pressure of its two sides, as long as the hydrotropic stimulus lasted. This disturbance of the equilibrium within the cell may be the direct stimulus perceived by the cell as a unit, which produces the differential growth of the opposite sides of the root. The means by which this reaction is carried out are just as mysterious as they are in phototropism and geotropism. It was shown that the root-tip is more sensitive than the rest of the root. This may be connected with the absence of the vacuole in the embryonic cells of the growing point. Their presence would facilitate the establishment of an equilibrium and diminish the effect of a difference in osmotic pressure.

Miss Eckerson (11) has found that, when roots bend after exposure to a difference of temperature on their opposite sides, the cells of the concave side are more permeable than those of the convex side. From this she concludes that heat affects the permeability directly, and that the consequent turgor change offers a mechanical explanation of the curvature. Pfeffer<sup>2</sup> has shown, however, that temperature can never exercise any marked direct effect on turgidity. Marked alteration taking place in either the osmotic pressure or in the diosmotic properties of the protoplast must be a reaction on the part of the cell to a stimulus, since such changes are regulated by the vital activity of the organism. Moreover, there is an exact parallel between the condition found by Miss Eckerson and that resulting from exposure to a hydrotropic stimulus. The difference of permeability would occasion a flow of water across the root from the concave to the convex side, and a disturbance of the equilibrium within the cells would thus be effected in exactly the same way as by the difference of osmotic pressure in hydrotropically stimulated roots. Therefore the resulting bending would be a reaction to a stimulus identical with that occasioning hydrotropic reactions, and not a mechanical curvature resulting from differences of turgidity. Consequently, so-called thermotropic reactions are largely due to the vital factor discussed in this paper. On account of the environmental conditions to which roots are exposed in thermotropic experiments, the mechanical factor mentioned above cannot play a part.

Since it seemed probable that hydrotropism was in the last analysis osmotropism, the following experiment was made. Two solutions of 1.15

<sup>1</sup> Pfeffer's Physiology of Plants, vol. i, p. 138. Second English edition. Oxford, 1914.



per cent. agar were prepared, and to one of them NaCl was added in the proportion of 1.015 gr. per 100 c.c. The two solutions were poured into tumblers and allowed to solidify. The agar blocks were then removed, cut smoothly down the middle, and the half blocks paired, so that each tumbler held one half with and one without salt. Of 20 roots placed between these half blocks, 16 were bent into the salt-free block after seven hours. This reaction would naturally be considered chemotropic, but its analogy to hydrotropism and osmotropism is striking, and raises the question whether many of the results obtained and classed as chemotropic are not in reality of an osmotic nature.<sup>1</sup>

#### SUMMARY.

1. Roots of *Lupinus albus* are always positively hydrotropic.
2. Hydrotropic reactions occur in roots only between 80 and 100 per cent. relative moisture.
3. The minimum moisture difference to which roots react at 20° C is a fall of 0.2 per cent. per cm.; the optimum is 0.4 per cent. per cm.; the maximum is 0.5 per cent. per cm.
4. Under optimum conditions six hours elapse before hydrotropic reaction is visible in roots. A presentation period could not be determined.
5. The hydrotropic sensitivity of roots resides chiefly in the tip, but also to a lesser degree above the root-tip.
6. Two factors determine the reaction of the root to a hydrotropic stimulus; one mechanical and the other vital. The intensity of the reaction varies inversely as the former and directly as the latter. When the stimulus is weak, the vital factor predominates; when too intense (above the maximum) the mechanical factor determines the reaction.
7. Hydrotropism is not a special case of traumatropism, but is probably equivalent to osmotropism.

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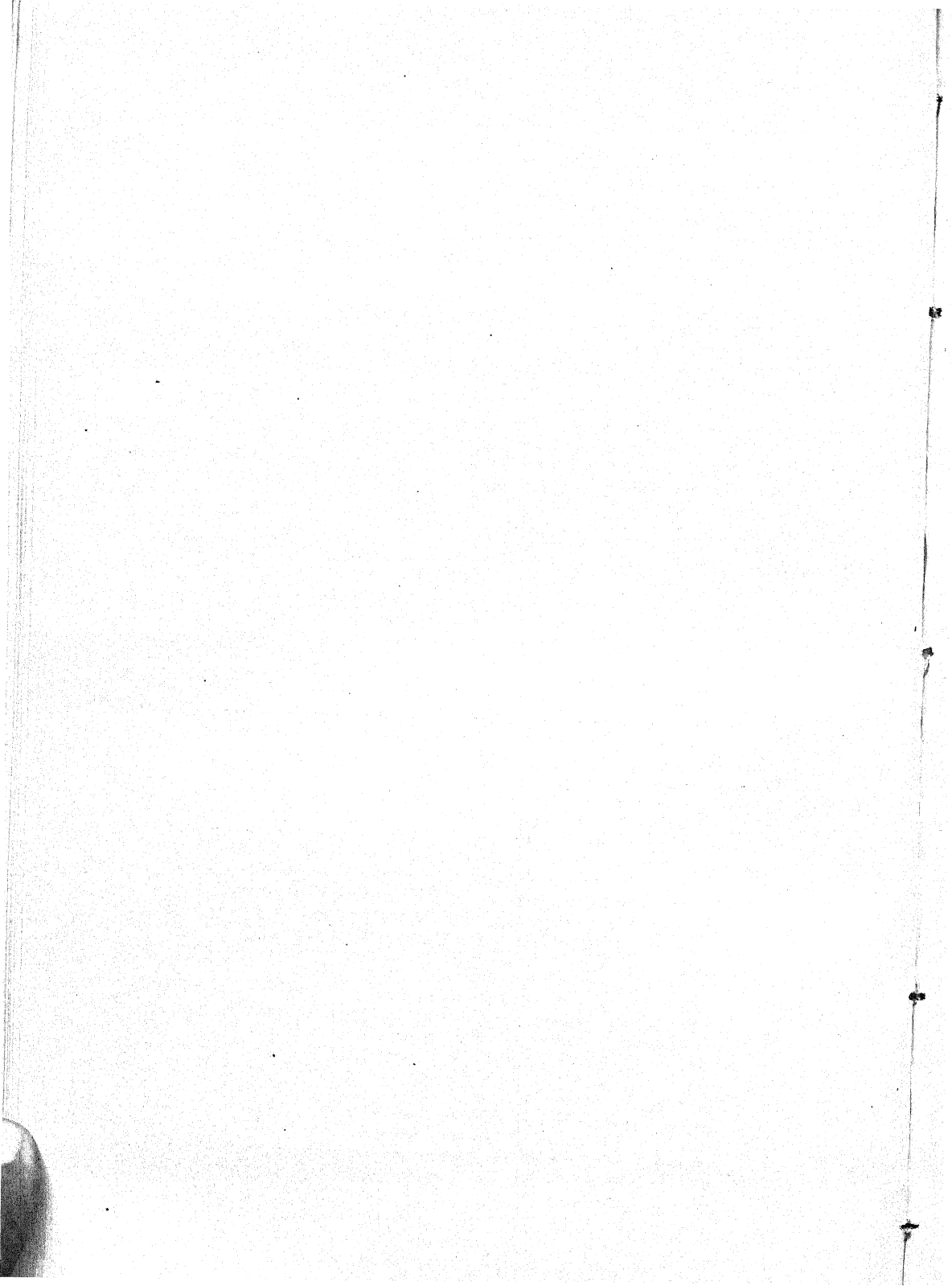
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<sup>1</sup> Cf. Porodko, Über den Chemotropismus der Pflanzenwurzeln, Jahrb. f. wiss. Bot., Bd. xlix, 1911, p. 347, Tabelle 17.

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# On Chromatin Extrusion in Pollen Mother-cells of *Lilium candidum*, Linn.

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With Plate XV.

THE improved technique of modern cytological research has revealed many interesting nuclear phenomena, one of the most significant of which is the process of 'chromatin' extrusion from the nucleus of one pollen mother-cell into the cytoplasm of an adjacent cell during various stages in the heterotype division. Although this condition had previously been noticed by several botanists, no importance was attached to it before 1909, in which year Miss Digby published a very complete account of the extrusion of 'chromatin' bodies from the pollen mother-cells of *Galtonia candicans* (4). In every respect the cells in this plant appeared to be quite normal, while the fixation was perfect. In two more recent papers the same author (Digby (5, 6)) calls attention to a similar protrusion of 'chromatin' into adjacent mother-cells of anthers of *Crepis taraxacifolia* and *Primula kewensis* (type) respectively.

In the course of his investigations on the cytology of various races of *Oenothera*, Gates (10) discovered and gave a full description of a process of 'chromatin' extrusion during the synaptic stages of the pollen mother-cells of *Oenothera gigas* and of *Oenothera biennis*, the extrusion taking place along definite cytoplasmic connexions between adjacent mother-cells. Describing the subsequent behaviour of the extruded material, this author (l. c., p. 935) states that 'the extruded chromatin accumulates in a mass after passing through the cell-wall. A clear liquid appears around these masses, and a membrane delimits the clear area from the cytoplasm, forming what I have called a pseudo-nucleus. Later, these masses loosen up, and acquire an appearance very similar to a spireme. The membrane afterwards disappears and the extruded chromatin finally appears to be

incorporated with the surrounding cytoplasm.' For this process Gates has proposed the term 'cytomixis'.

Since a similar condition has never been recorded for *Lilium*, although this genus has been the subject of much cytological investigation, it seemed that the phenomena observed in our preparations were of sufficient interest to justify the present paper.

#### METHODS.

The material used in this investigation was fixed in chromo-aceto-osmic acid (strong formula of Flemming), or in Hermann's solution, or in acetic alcohol (1 part glacial acetic : 3 parts absolute alcohol). A variety of cytological stains were employed, including Flemming's triple; Heidenhain's haematoxylin with a counter-stain of Bordeaux red or orange G; gentian violet and orange G; safranin; and the methylene blue, safranin, orange tannin combination of Breinl. For determining the nature of the cell-walls methylene blue, ruthenium red, and Congo red were found useful.

#### DESCRIPTION.

During synapsis, when the 'chromatin' typically assumes the form of a dense more or less homogeneous mass, in which it is almost impossible to distinguish individual spireme threads, the nucleus almost invariably takes up an excentric position in the cell, frequently appearing as if pressed against the gelatinous membrane which separates the mother-cells at this stage, and in which distinct perforations have been demonstrated by Körnicke (14), Gates (9), and Digby (4). It is impossible to reconcile Schaffner's (18) conclusion that the condition of synapsis is an artifact with the results of many experienced cytologists, who have repeatedly described and figured such a condition of the nucleus. Moreover, several observers have actually seen a typical synaptic phase in living material of both animals and plants.

From the framework of the nucleus globules of a substance which readily takes up so-called 'chromatin' stains are budded off. These globules (= 'chromatin bodies' of Digby) vary considerably in size and number and, according to our observations, always penetrate the cell-wall (Figs. 1-5), probably in the neighbourhood of the cytoplasmic connexions. At this stage the cell-wall is very thin and stains deeply with ruthenium red, thereby indicating its pectose nature. Congo red and other 'cellulose' stains gave negative results. This extrusion usually takes place simultaneously and in the same direction from all the mother-cells of a locus, but a few loculi were noticed in which the mother-cells at either end were discharging towards the centre, whilst the cells occupying a position near the centre of the locus retained the typical condition of complete synapsis.

In this way, then, the globules reach the cytoplasm of an adjacent mother-cell, where they at first appear as smaller or larger roundish deeply staining masses, each one of which retains its connexion with the synaptic knot of the parent nucleus by means of a very fine thread passing through the cell-wall (Figs. 1-5). These connecting threads, which persist for a considerable period, judging from the frequency with which they are found, also take up 'chromatin' stains, hence their presence can easily be demonstrated in preparations stained with iron-haematoxylin, or with the combination stains of Flemming or of Breinl. The extruded globules frequently assume a pear-shaped outline and occasionally 'secondary globules' are protruded from their periphery (Fig. 5). These bodies are always surrounded by a perfectly clear zone; the precipitation-membrane delimiting this clear space from the surrounding cytoplasm appears to be of the same nature as that which surrounds the nuclear vacuole and in both cases is very indistinct. In preparations treated with the above-mentioned cytological stains the nuclear vacuole appears to be bounded by a close aggregation of cytoplasmic fibrils (Figs. 1 and 4).

At a somewhat later stage, when the bodies have lost their connexion with the parent nucleus, they appear, in carefully stained preparations, as isolated deeply stained globules scattered throughout the cytoplasm of the invaded cell. Great difficulty was experienced in following the subsequent history of the extruded material, all trace of which is very quickly lost. It appears to be ultimately absorbed by the cytoplasm of the invaded cell.

In two anthers which had been fixed in Hermann's solution the condition of excessive 'chromatin' extrusion shown in Fig. 3 was observed.

These bodies are also given off while the nucleus is coming out of synapsis and during the inception of the 'hollow-spireme' stage. That these bodies are actually extruded during the later synaptic stages, and are not merely the remains of those given off at complete synapsis, is shown by the fact that at these later stages the globules are usually small and rounded while the connecting threads are very well defined. Moreover, these connecting threads always persist until the walls of the mother-cells begin to separate. During these later stages of meiosis the elimination of nuclear material is usually less pronounced (Figs. 4 and 6). The extruded material often presents a beaded or granular appearance (cf. Digby (4), Pl. XXXIV, Fig. 16), which is probably due to a difference in 'chromatin' concentration in the substance of the 'bodies', but nothing approaching the condition of a spireme, as described by Gates for *Oenothera gigas*, has been observed in *Lilium candidum*.

'Chromatin' extrusion into an adjacent tapetal cell was never observed in our preparations, although lateral extrusion from one mother-cell into two neighbouring mother-cells, or lateral extrusion from two mother-cells

into the cytoplasm of a single mother-cell, was frequently found; Gates's (10) (l. c., p. 917) explanation that this is due to the complete absence of cytoplasmic connexions between the pollen mother-cells and the cells of the tapetum is probably correct.

#### RÔLE OF THE NUCLEOLUS.

Apart from the behaviour of the nucleolus, the descriptions of 'chromatin' extrusion in *Galtonia* and *Oenothera* respectively show a close agreement. In *Galtonia*, active nucleolar budding is described by Miss Digby; in *Oenothera*, on the other hand, the nucleolus, according to Gates, takes no part in the extrusion. For this reason, the behaviour of the nucleolus in *Lilium candidum* was studied with special care; we were nevertheless led to the conclusion that it takes no part whatever in the process under discussion. That is to say, during the various synaptic phases the nucleolus (or nucleoli, since two are occasionally found in the same nucleus) retains its definite boat-shaped or spindle-shaped outline. Vacuoles were sometimes noticed in the substance of the nucleolus (Figs. 1 and 4), but no sign of nucleolar budding was observed.

#### DISCUSSION.

Körnicker (14) described the peculiar appearances presented by a number of preparations of *Crocus vernus*. A definite extrusion of chromatic substance from the pollen mother-cell nucleus and its passage through the cell-wall into the cytoplasm of an adjacent mother-cell were observed. Körnicker attributed this phenomenon to an abnormal condition of the anther at the time of fixation, and states (l. c., p. 24): 'Am wahrscheinlichsten ist, wie ich glaube, die Annahme, dass es sich um den Ausdruck einer Alteration handelt, welche die Pollenmutterzellen erlitten haben bevor die Antheren den jungen Blütenanlagen entnommen wurden, und deren Ursache auf folgende Bedingungen zurückzuführen ist, unter welchen sich die Antheren in der Pflanze befanden.' He maintains that the release of pressure on artificially opening the bud, which to a certain extent compresses the anthers, may allow a sudden expansion of the latter whereby the protoplasmic connexions between the pollen mother-cells are broken. In this way a slight wound stimulus is given to the cells.

The interesting experiments of Miehe (15), Hottes (13), and Schrammen (19) may be quoted in support of this hypothesis. Miehe clearly showed that a transference of nuclear material from one cell to an adjacent cell can take place as a direct response to certain traumatic stimuli. He figures (l. c., Taf. xi, Figs. 2 and 3) such a process in epidermal cells of a young leaf of *Allium nutans*. But the fact that Miehe obtained his results in typical vegetative cells, the nuclei of which were probably in the 'resting' condition, must be taken into account when the two processes are compared.

Hottes and Schrammen subjected various regions of the vegetative tissues of *Vicia Faba* to sudden changes in temperature; subsequent examination of the parts thus treated revealed certain anomalous nuclear conditions comparable in many respects with those obtained by Mische in *Allium nutans*.

In 1905 Gregory (11) published a brief account of the cytology of a sterile race-hybrid of *Lathyrus odoratus*, in which a similar process was figured (l. c., Pl. II, Figs. 16 and 17). However, Gregory erroneously described this phenomenon as an incomplete or abnormal division by constriction of the pollen mother-cells. He states further that such cells always degenerate, and that the sterility in these plants is confined to the male organs.

According to Rosenberg (16, 17), nuclear material is frequently pressed through the wall of a pollen mother-cell during synapsis, both in *Crepis virens* and in *Drosera longifolia*. No importance was attached to this phenomenon, which was thought to be the result of faulty fixation.

A similar condition was observed in the pollen mother-cells of *Vicia Faba* by Fraser (8), who remarks (l. c., p. 635) that the extruded 'bodies' 'seem clearly related to the incidence of an abnormal condition'.

This phenomenon has therefore in turn been regarded as :

1. the result of an abnormal physiological condition of the anther at the time of fixation (Körnicke);
2. an artifact (Rosenberg);
3. a sign of the subsequent degeneration of the mother-cells concerned (Gregory, Fraser).

There remains a fourth explanation, namely, that this process represents a perfectly normal condition of the pollen mother-cell during synapsis. This, in our opinion, is the correct explanation, for there is certainly no evidence, at least in *Lilium candidum*, to indicate either that this condition is an artifact or that it foreshadows the degeneration of the mother-cells.

Miss Digby found that after the nucleus had returned to the centre of the cell, the latter presented a perfectly healthy appearance ((4) Pl. XXXIV, Fig. 17), although the disintegrating fragments of the 'bodies' could still be identified in the cytoplasm as bright refractive granules.

Moreover, Gates (10) (l. c., p. 918) states distinctly that in *Oenothera gigas* 'the nuclei appear perfectly normal after the extrusion has taken place'.

The data now at our disposal are sufficient to justify the conclusion that future research will demonstrate the general occurrence throughout the Plant Kingdom of such an elimination of nuclear material as a normal phase in meiosis. It may represent an excretion of waste products from the nucleus at a time when its metabolism is subject to sudden profound changes. It is well known that the metabolic processes of the nucleus are



very active during the various phases leading up to and concerned with the reduction division. Many interesting cases of 'chromatin' extrusion into the cytoplasm of the same cell have recently been recorded. This condition appears to be widespread amongst both animals and plants. It has been observed in a number of plants by von Derschau (2, 3),<sup>1</sup> in various Ferns by Farmer and Digby (7), in *Crepis virens* by Digby (6), and in *Helvella* by Carruthers (1), whilst the peculiar process of nuclear gemmation observed by Griggs (12) in *Synchytrium* exhibits many features in common with the above.

We are not yet in a position to decide whether the cases cited above bear any relation to the phenomena described for *Lilium candidum*; it is therefore advisable to leave it an open question as to whether they should come under the same category.

Amongst the lower animals, especially the Protozoa, an elimination of nuclear material has been observed and described by many authors. Here again the phenomena observed probably represent an excretion of waste products from the nucleus, and are no doubt analogous with the processes described above.

The condition of excessive 'chromatin' extrusion found in two anthers of *Lilium candidum* suggests the degeneration of the mother-cells, and consequently the abortion of the pollen grains; against this it can be urged that although a large number of anthers showing later stages in the development of the pollen were examined, not one of these presented the appearances usually associated with pollen sterility.

#### SUMMARY.

1. A process of 'chromatin' extrusion from pollen mother-cell nuclei into the cytoplasm of adjacent mother-cells is described for *Lilium candidum*.
2. This process takes place during the synaptic and 'hollow spireme' stages.
3. The nucleolus takes no part in the extrusion.
4. The authors regard this phenomenon as a normal condition of meiosis, the extrusion of nuclear material (i.e. waste products) being attributed to the active metabolism of the nucleus during the meiotic phase.

In conclusion we desire to express our thanks to Professor J. Bretland Farmer, F.R.S., for much helpful advice and criticism throughout the course of this investigation.

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EXPLANATION OF PLATE XV.

Fig. 1. Nucleus in synapsis showing extrusion of 'chromatin' into an adjacent cell. × 1,050.

Fig. 2. Nucleus in synapsis showing extrusion of 'chromatin' into an adjacent cell. Note 'chromatin' concentration in the largest extruded mass. × 900.

Fig. 3. Nucleus in synapsis showing excessive 'chromatin' extrusion. Note the isolated 'chromatin bodies'. × 1,200.

Fig. 4. Nucleus coming out of synapsis; shows early stage of 'chromatin' extrusion. Note vacuolate nucleolus. × 1,000.

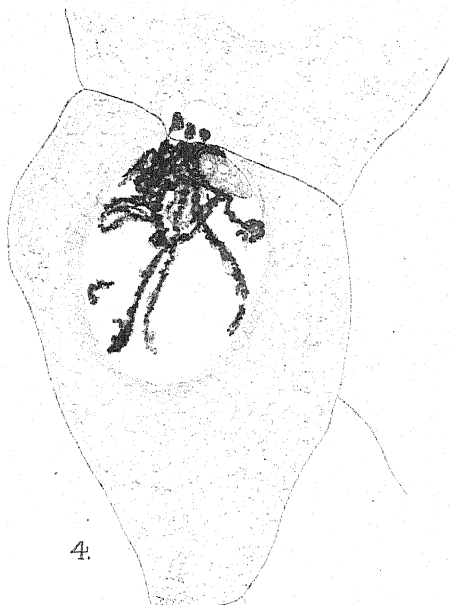
Fig. 5. Nucleus in synapsis showing extrusion of 'chromatin'. Note 'secondary processes' protruded from the larger 'bodies'. × 1,200.

Fig. 6. Nucleus in the 'hollow spireme' stage showing extrusion of 'chromatin'. × 1,000.

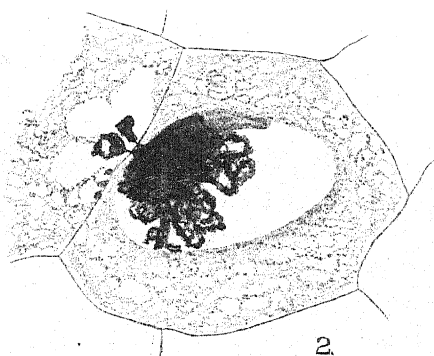




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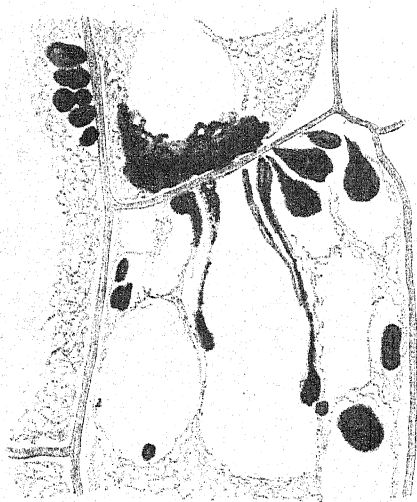
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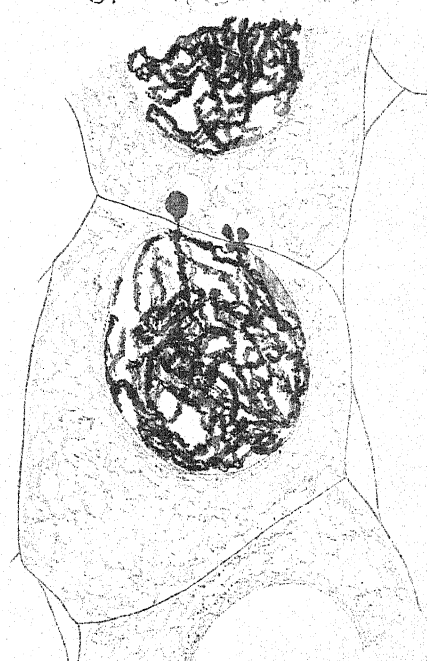
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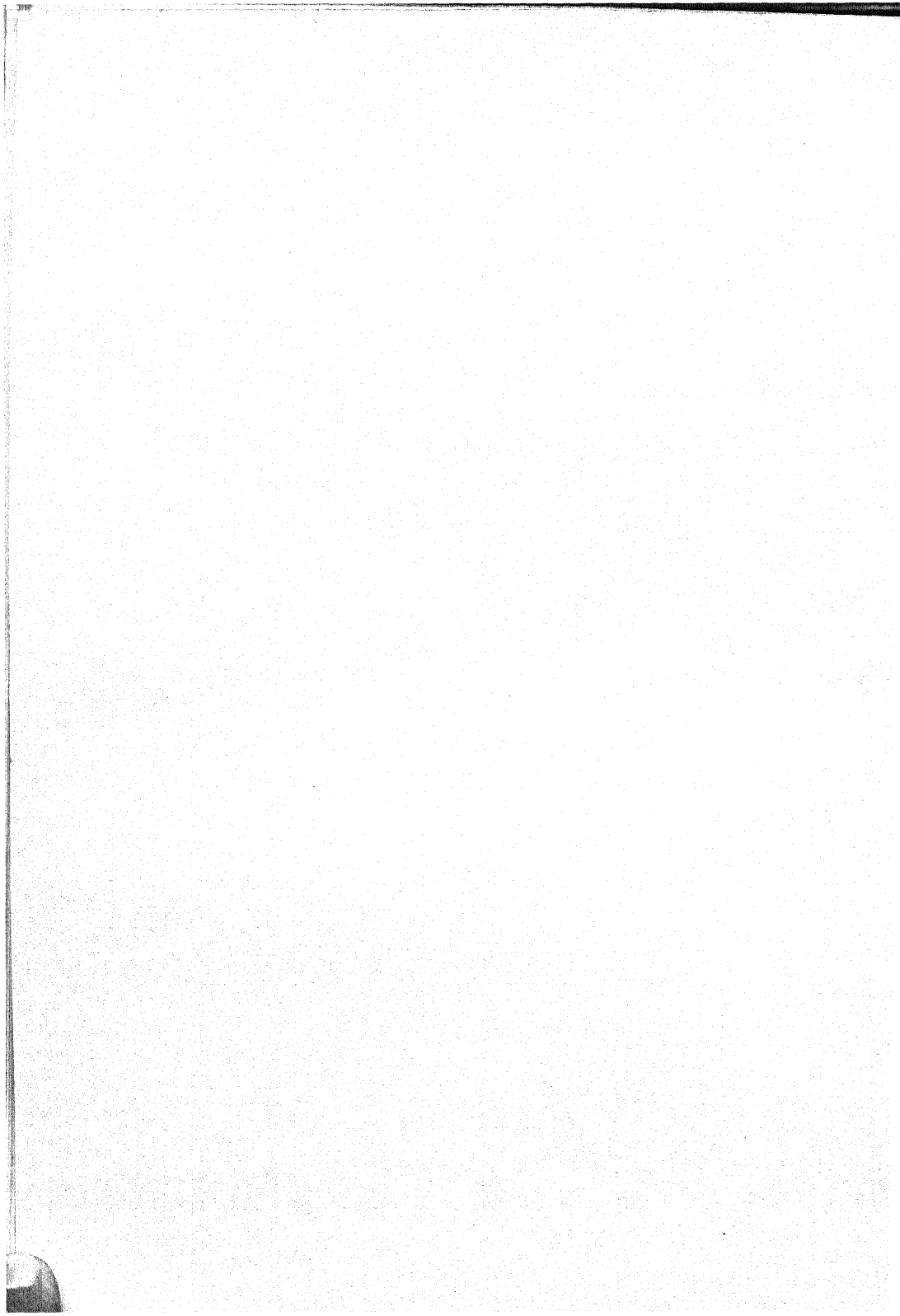


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# Nuclear Migrations in *Phragmidium violaceum*.

BY

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With Plate XVI.

DURING the last ten years much work has been done on the life-history of the Uredineae, and it has brought to light the fact that the development of the aecidiospores is initiated by the formation of binucleated cells at the base of the aecidium.

Blackman (1),<sup>1</sup> in 1904, was the first to show how this took place. He found that in *Phragmidium violaceum* the binucleate condition originated in the 'fertile cell' of the aecidium, and was the result of the migration into it of the nucleus of a neighbouring cell. He considered this to be a case of reduced fertilization, in which a vegetative cell takes the place of a normal male cell. He regarded the spermatia as male cells which had become functionless, and suggested that the sterile terminal cell might represent an abortive trichogyne.

In 1905 Christman (3) investigated the aecidial development of *Phragmidium speciosum*. He found that in this form the binucleate condition is brought about by the gradual breaking down of the walls separating two adjacent fertile cells, so that they fuse and eventually give rise to a row of binucleate aecidiospores. He considered this to be a conjugation of two equal gametes. The spermatia he regarded as gametophytic conidia. This type of fertilization was later interpreted by Blackman and Fraser (2) as a case of reduced fertilization in which two female cells associate. This second type of fertilization is thus brought into line with the earlier one found in *Phragmidium violaceum*.

Since this time a large number of forms have been investigated, and it has been shown in the majority of cases that fertilization takes place by the union of two similar cells. Such a mode of origin of the binucleate condition has been described for *Phragmidium speciosum* (3), *Melampsora Rostrupi* (5), *Phragmidium potentillae canadensis* (5), *Caeoma nitens* (12 and 13), *Triphragmium Ulmariae* (13), *Puccinia transformans* (13), *Puccinia*

<sup>1</sup> The numbers refer to the list of papers given at the end.

*Falcaria* (6), *Endophyllum sempervivum* (11), *Melampsora Linii* (8), *Puccinia Claytoniata* (9), *Uromyces Caladii* (3 and 9), *Puccinia violae* (9), *Puccinia angustata* (9), and *Puccinia fusca* (14). So far fertilization in the Uredineae by means of a migrating nucleus has been clearly observed only in *Phragmidium violaceum* (1), and with somewhat less clearness in *Uromyces Poae* (2) and *Puccinia Poarum* (2).

The far more common occurrence of the method of fertilization by the union of similar cells has led to some doubt being thrown on the importance of nuclear migration in the sexual process of this group. Olive (13) found nuclear migrations in *Triphragmium Ulmariae* and *Caecoma nitens*, but held them to be early stages of cell fusions, whilst Christman and Kurssanow, on the other hand, considered them to be pathological phenomena. Professor Blackman accordingly suggested to me the re-examination of the developing aecidium of *Phragmidium violaceum*, especially with a view to ascertain if cell fusions, as well as nuclear migrations, were to be found there.

The material was collected on Leith Hill and in the neighbourhood of Guildford in the spring of 1913 and 1914. Owing to the difficulty of distinguishing between *Phragmidium Rubi* and *Phragmidium violaceum* in the aecidial stage, teleutospores were gathered in the autumn from those blackberry bushes which had previously furnished the material of aecidia. The characters of the teleutospore were those of *Phragmidium violaceum*, Wint., the form investigated by Blackman (1).

Special attention was paid to fixation in view of Christman's suggestion that the nuclear migrations might have been the result of wounding during that process. The young aecidial patches are red in colour and were always cut with a sharp pair of scissors from leaves still on the plant, leaving a margin of green tissue round each infected area. The material was then put direct into the fixative. Flemming's strong solution, diluted with an equal quantity of water, was commonly used, penetration of the fluid being facilitated by the use of a small air-pump, so that the material sank in a few moments. Well-fixed material was also obtained by using in the same way Bouin's picro-formol. As a control, some satisfactory preparations were made by momentarily immersing the material in 30 per cent. alcohol before placing in the fixing fluid; this ensured the quick penetration of the fluid without the use of a pump. Material was also fixed in acetic alcohol.<sup>1</sup>

A very careful and prolonged search has been made for any indications of fusions between fertile cells of the young aecidium, but without success; no case of this type of fertilization was found. Migrations of nuclei, such as were previously described by Blackman, occur regularly. The nucleus invariably passes from a vegetative to a fertile cell; sometimes the cells concerned belong to the same hypha and the nucleus passes from below upwards (Pl. XVI, Fig. 9), and at other times the nucleus comes from a neighbouring

<sup>1</sup> Nuclear migrations were found in material fixed in all these various ways.

hypha and passes in laterally (Fig. 2). In no case was a migration found from one fertile cell to another. In very young aecidia the migrations were found to occur in the middle region of an aecidium, whilst in rather older examples cells of this region were already binucleate, and then the migrations were found only in the cells immediately outside these. Sometimes the nucleus passes through a very small pore in the wall (Figs. 1 and 4), and thus becomes greatly constricted (cf. Blackman (1), Figs. 67, 68). More commonly, however, the pore is rather larger, and the nucleus is only slightly constricted during its passage and corresponds to Blackman's Fig. 66; such cases are shown in Pl. XVI, Figs. 2, 3, 5, 6, and 7. In a few cases the hole was of considerable size, as shown in Figs. 8 and 9. When it was found that the nuclei sometimes pass through a large hole on the cell-wall, it seemed unlikely that such holes would be later obliterated, and a careful search was made amongst the older binucleate cells, with the result that a few cases were observed, and two of these are shown in Pl. XVI, Figs. 10 and 11.

The cells from which the nuclei have migrated gradually lose their cytoplasm and become almost or quite empty; they form a fairly conspicuous layer surrounding the bases of the old fertile cells. Pl. XVI, Fig. 13, is a semi-diagrammatic drawing of the middle of a young aecidium, the fertile cells are binucleate, and below them is the layer of empty cells. Part of the same aecidium, but nearer to the periphery, is shown in Fig. 12 where the fertile cells are as yet uninucleate and have no empty cells at their bases. In thick sections of older material the empty cells form a conspicuous layer; Fig. 14 is a semi-diagrammatic representation of such a preparation, showing the young aecidiospores, the binucleate fertile cells, a layer of empty cells, and below that a mass of uninucleate hyphae ramifying in the tissue of the host.

In spite of a careful search, fusion between two fertile cells was never observed, but no less than twenty-eight cases of migration of a vegetative nucleus into a fertile cell were found. It is also important to note, as stated earlier, that in fairly young aecidia the migrations are found to occur only in connexion with the cells immediately peripheral to the central mass of binucleate fertile cells. This special localization of the 'migrations' and the absence of lateral fusion of fertile cells are in themselves sufficient to show that the passage of a vegetative nucleus into a fertile cell is the normal method of origin of the binucleate condition in this form. Migrating nuclei always pass from a vegetative to a fertile cell, and no cases were found of nuclear migrations between fertile cells or between vegetative cells. It may be mentioned that the paraphyses towards the periphery of the aecidium are often multinucleate, and nuclear divisions occur frequently in them.

Christman has put forward a very interesting view as to the morphology of the aecidium and the phylogeny of the group generally. He points out



that, from analogy with other groups, the gametophyte generation should be the primitive generation, and therefore that those forms with the simplest sporophyte generation—that is to say, the lepto- and micro-forms—are the most primitive. He regards the various types of spores as homologous, and considers that in the lepto- and micro-group the gametophyte bears the gametes and produces the fusion cell. The point at which the cell fusions occur has receded further and further from the teleutospore, the sporophyte generation becoming more elaborate, till, in the higher groups, an aecidium has been introduced and the eu-forms appear. The 'fusion cell' he regards as the product of two *isogametes* which have formed a zygospore like that of the moulds, but, unlike them, has no resting stage and produces many spores. The spermatia he regards as gametophytic conidia.

The main objections to this view are (1) that it ignores the phenomena described for *Phragmidium violaceum*, assuming that they are due to some pathological cause, and (2) that it offers no adequate explanation of the spermatia.

The observations recorded in this paper show the untenability of the view that nuclear migrations in the aecidium of *Phragmidium violaceum* are pathological in nature. Also one would hardly expect to find conidia which are apparently functionless produced at the same time as the very effective aecidiospores; and, as has been pointed out by Blackman, the relative proportion of nucleus and cytoplasm exhibited by the spermatia is quite out of keeping with what is known of conidia.

The alternative hypothesis is that put forward by Blackman in 1904. He regards the Uredineae as a group in which a great variety of reduced forms occur. He considers that they are derived from an ancestor with a typical sexual process, the male cells being now represented by the spermatia and the female by the 'fertile cells'; these latter were provided with a trichogyne which now possibly exists as a 'sterile' or 'buffer' cell. According to this interpretation, all these Uredineae which have at present been investigated have a reduced type of fertilization, *Phragmidium violaceum* being fertilized by a vegetative instead of a male cell, whilst in *Phragmidium speciosum* reduced fertilization is effected by means of female cells. The two types of fusion found in the Uredineae are thus considered to be heterogamous instead of isogamous, and the group is regarded as showing relationship with the Florideae rather than with the Zygomycetes.

It is, of course, very difficult to decide between these two views. There are at present no data as to whether the heterogamous or isogamous union in the aecidium is the more primitive. If the nuclear migrations of *Phragmidium violaceum* are to be looked upon as reduced in comparison with the isogamous unions which have been described for so many aecidia, then it is still possible to homologize, with Christman, the aecidium and the primary uredospore cell. On the other hand, the fact that the Caeomas of

closely allied forms show two very different types of fertilization certainly lends support to the view that both are reduced, being derived probably from an earlier normal sexual process of fertilization by spermatia. If we agree that the peculiar fertilization processes of the aecidium and the existence of the spermatia are sufficient evidence that the more primitive sexual organs are to be found in the aecidium, then we must assume that this type of spore form is the oldest. This is the essential point of Blackman's view as opposed to Christman's. It is not necessary to assume that the eu-forms as they occur at the present day are more primitive than, for example, the brachy- or micro-forms. It is possible that these forms were reduced from a primitive aecidium-bearing form independently of the eu-forms, by the loss of the aecidium and the shifting forwards in the life-history of the point of nuclear association. On the other hand, the complex eu-forms may have developed independently with further elaboration of the life-history, but without loss of the aecidium.

#### SUMMARY.

1. A re-examination of *Phragmidium violaceum* completely confirms Blackman's observation that fertilization is brought about by the migration of a vegetative nucleus to a fertile cell.
2. No other mode of origin of the binucleate cells was observed.
3. The size of the pore through which the nucleus passes is very variable, sometimes being as much as  $3\ \mu$  in width. Cells were found in which the pore was visible after the nucleus had migrated through it.
4. A layer of more or less empty cells occurs immediately below the binucleate fertile cells, and is made up of those cells from which the nuclei have migrated.
5. That the nuclear migrations are not pathological in nature is shown by the facts that:
  - (i) They occur in regular sequence from the middle to the periphery of the aecidium.
  - (ii) They are not found in the paraphyses at the periphery of the aecidia where the cells are nearer to the wounded surface.
  - (iii) They are found in material fixed in various ways.

It is with great pleasure that I record my thanks to Professor Blackman for his valuable help and criticism.

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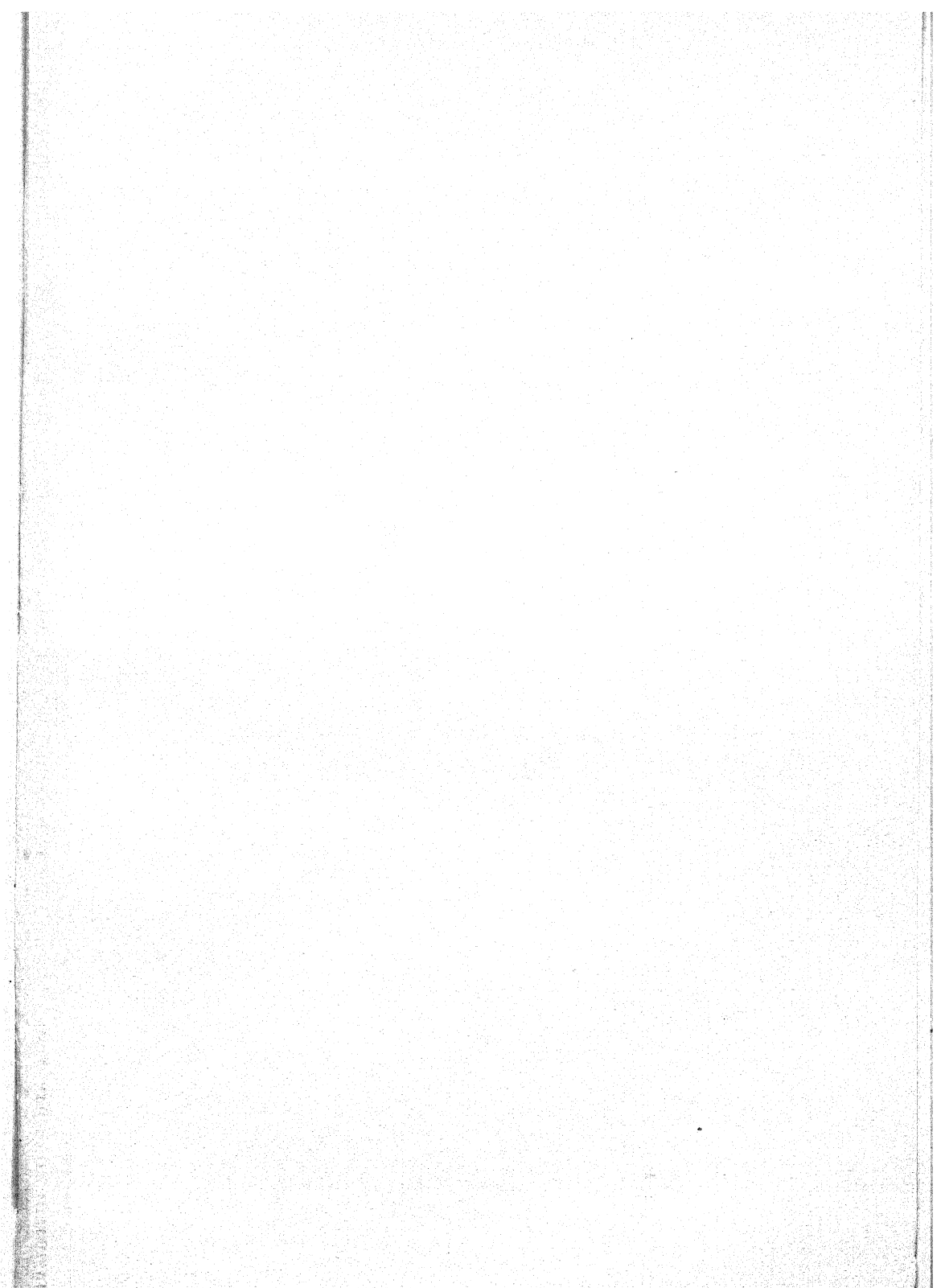
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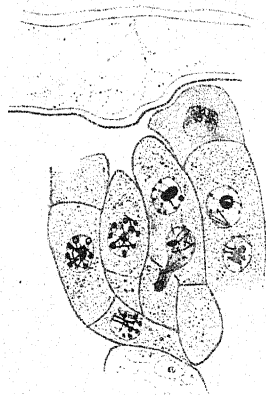
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14. PAVOLINI: L'Ecidio della *Puccinia fusca*. Bull. Soc. Bot. Ital., 1912.

## DESCRIPTION OF PLATE XVI.

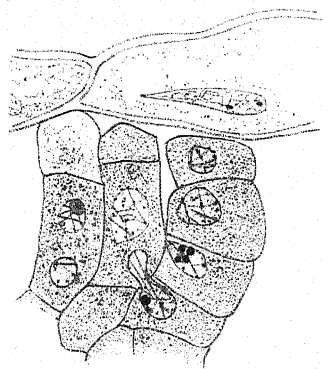
Illustrating Miss Welsford's Paper on *Phragmidium violaceum*.

- Fig. 1. Migration of nucleus. The sterile cell has been cut away.  $\times 1,300$ .
- Fig. 2. Migration of nucleus between the cells of separate hyphae.  $\times 1,300$ .
- Fig. 3. Ditto.  $\times 1,300$ .
- Fig. 4. Migration of nucleus from vegetative cell to fertile cell immediately above it on the same hypha.  $\times 1,300$ .
- Fig. 5. Ditto.  $\times 1,300$ .
- Fig. 6. Migration of nucleus from vegetative cell of one hypha to fertile cell of another.  $\times 1,300$ .
- Fig. 7. Ditto.  $\times 1,300$ .
- Fig. 8. Ditto. In this case the nucleus is passing through a very large pore between two hyphae.  $\times 1,300$ .
- Fig. 9. Ditto. The nucleus passing through a large pore between two cells of the same hypha.  $\times 1,300$ .
- Fig. 10. A binucleate cell showing the pore through which the nucleus has passed.  $\times 1,300$ .
- Fig. 11. A binucleate cell showing the pore through which the nucleus has passed.  $\times 1,300$ .
- Fig. 12. Semi-diagrammatic drawing of a young aecidium (peripheral region). The fertile cells are uninucleate, and no empty cells can be seen.  $x$  = host cells.  $\times 500$ .
- Fig. 13. Semi-diagrammatic drawing of the same aecidium as that shown in Fig. 12, but in the median region. Here the fertile cells have become binucleate and empty cells can be seen near their bases.  $x$  = host cells;  $e$  = empty hyphal cells.  $\times 500$ .
- Fig. 14. Semi-diagrammatic drawing of a nearly mature aecidium, showing the young aecidiospores, the binucleate fertile cells, the layer of empty cells, and the uninucleate hyphae ramifying amongst the host cells.  $\times 500$ .

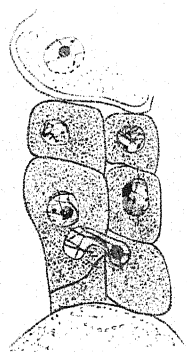




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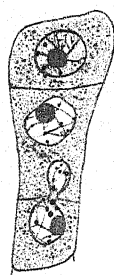
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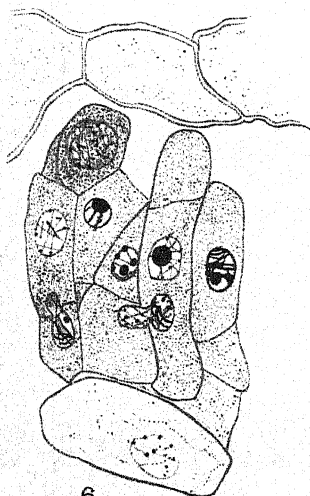
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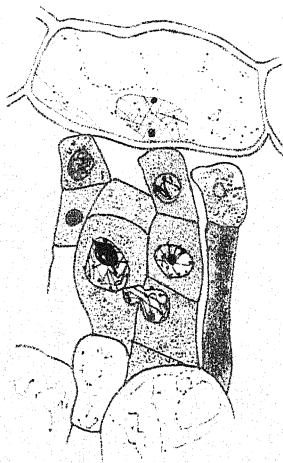
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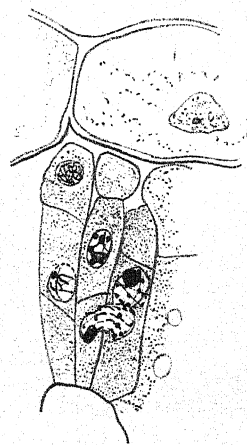
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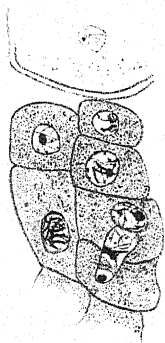
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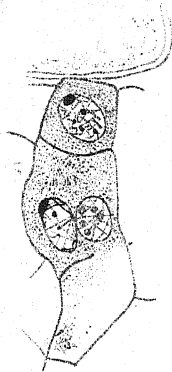
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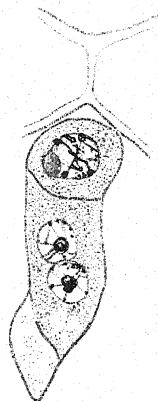
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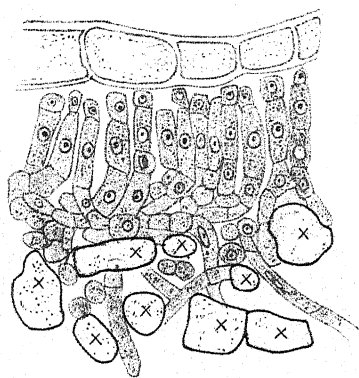
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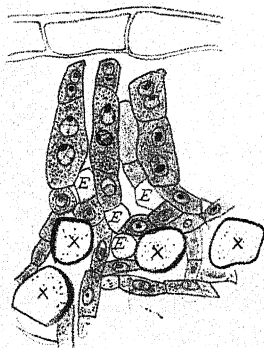
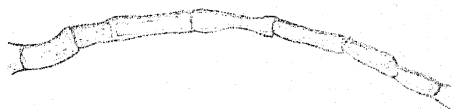
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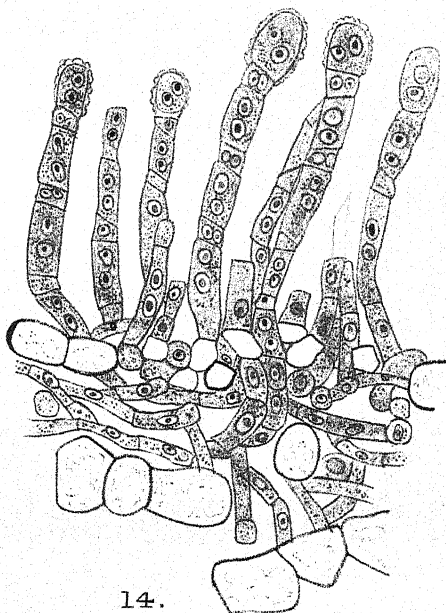
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Huth lith et imp.



## The Origin of the Tristichaceae and Podostemaceae.

BY

J. C. WILLIS, M.A., Sc.D.,

*Director of the Botanic Gardens, Rio de Janeiro.*

AS is well known, and will be considered in detail in another paper, the place to be assigned to these families in the Natural System has long been a matter of dispute. They have no nearly allied family, unless possibly Hydrostachydaceae, which live in similar conditions, and were once united to them. For the time being the dispute has ceased, since Warming placed them in the same order with the Saxifragaceae, with which they have in common a thick placenta and numerous ovules. I may remark, however, that I think they are just as near to the Nepenthaceae, which belong to another order, and probably to other families as well. It will help considerably in treating this difficult question, as well as in dealing with their evolution, if we consider in some detail their probable mode of origin.

These plants, it may be well to explain, are all water plants with a mode of life which in the flowering plants is unique to them and to the Hydrostachydaceae. With the exception of one species in the southern United States they are confined to the tropics and sub-tropics, but are very widely distributed, reaching from Java by India and Ceylon to Egypt and Madagascar, tropical and South Africa, South and Central America, and to Mexico and the United States. They live only attached to rocks, usually smooth and waterworn, or other firm substratum, in the beds of mountain streams, where the water moves rapidly over them, so rapidly that it is often full of bubbles of air. If they be placed in standing water they soon die, and they are absolutely incapable of existence on a sandy or muddy substratum.

They cling to the rocks by means of root-hairs, or more often by haptera, special adhesive organs of probable root nature, which usually appear as exogenous protuberances on root or shoot, bend downwards to the rock, and there cling by flattening out and secreting an adhesive substance.

These plants are purely vegetative during the period of high water-level due to the rains. As the water drops towards the close of the rains, they form their flowers, which open as the air touches them. The seeds quickly



ripen and are shed on the rocks, where they germinate with the rise of water at the beginning of the rains. The original plants usually die, but may rejuvenesce if there be an early rise of water.

To go on now to the question of the origin of these plants in the first place, what is their origin regarded merely as Angiosperms, which they undoubtedly are? Have they, as some writers think, descended continuously in water from aquatic pre-Angiosperms, or have they sprung from the general tree of the Angiosperms, which it is generally conceded began upon land? I think that there can be no doubt that the latter supposition is correct. If they were an independent survival of pre-angiospermous water plants, one would expect to find their embryology very different from what it actually is, for, as Warming,<sup>1</sup> Went,<sup>2</sup> and Magnus<sup>3</sup> have shown, it is typically dicotyledonous. I think that on this ground alone they must be regarded as descendants of the general tree of the Dicotyledons.

If for the first time one saw any of their flowers without knowing from what sort of plant they came, one would never for a moment imagine them to be the flowers of water plants. They are typical simple mono- or a-chlamydeous dicotyledonous flowers, sometimes ento-, sometimes anemophilous, and always, unless they happen to be cleistogamic, awaiting the fall of the water-level to expand in the air on the dry rocks, and be pollinated there. They do not look in the least like the flowers that one would expect in plants with a long line of hydrophytic ancestry, leading back to ante-floriferous days.

The fruit again is typically a land fruit. Only in the most highly modified species of the whole family—*Farmeria metzgerioides*—does it show any special suitability to the peculiar aquatic mode of life of these plants. Never, one would imagine, would a primitively aquatic family possess minute seeds in a capsule that only dehisced in dry air. Never would those seeds possess an outer layer of cells that became mucilaginous on wetting.<sup>4</sup> Nor would the seedlings be so badly provided with holdfasts that they were constantly washed away. There is no disputing the evidence that these peculiar families are the descendants of other Dicotyledons, and of Dicotyledons that lived on land, even if their immediate ancestors were water plants.

The next question is, Where did their immediate ancestors live? Was it (1) on land or (2) in water, and in the latter case were they water plants (a) of the reaches between the rapids and waterfalls in which the Tristichaceae and Podostemaceae live; (b) of moving water of the rivers of the plains or low country; or (c) of ponds or marshes?

<sup>1</sup> Warming: Familien Podostemaceae II. Kgl. Dansk. Vidensk. Selsk. Skr., 6 Række, ii, 1882.

<sup>2</sup> Went: Unt. über Podostemaceae. Verh. Koninkl. Akad. Amsterdam, xvi, 1910.

<sup>3</sup> Magnus: Die atypische Embryonalentw. d. Podostemaceen. Flora, cv, 1913, p. 275.

<sup>4</sup> This has often been described as an adaptation for clinging to the rock, by people who have forgotten that with another rise of water the seeds once more become loose and are washed away.

In the first place, to consider the possibilities of their having been water plants at all, there are several facts which are opposed to this hypothesis. To begin with, their seeds, with one or two exceptions among the most modified species of the orders, but with none among the more primitive, are all alike—very numerous, minute, exalbuminous, with an outer coat of cells which become mucilaginous on wetting, and contained in capsules which open only in dry air. These seeds are singularly ill adapted to the mode of life which characterizes these families. They fall upon the dry naked rocks within a few days of the beginning of the dry season, which in most of the districts where these families grow lasts for several weeks. Before the end of it the enormous majority will have blown away, and once off the rocks their chance of reaching suitable spots for growth is practically nil. But a few remain, and when the water rises, the bulk of these will wash away. In the case of plants of *Hydrobryum olivaceum* or *Laxia seylanica*, which I estimated to bear (where well grown) from 20,000 to 30,000 seeds on an average, the number of seedlings—or rejuvenescences—which may arise is rarely more than two or three, sometimes as many as ten, often enough one or even none. Were it not for rejuvenescence of the old thallus, one gathers the impression that the survival of some of the highly modified species like these would be problematical.

But even when they have actually germinated, many of the seedlings are washed away, and I do not think that it is much exaggeration to say that six weeks after germination each parent plant is usually represented by not more than one or two young ones.

But now, as these ill-adapted seeds are found throughout the families, except in a very few of the most modified species, it cannot be a stretch of probability to suppose that they are a legacy from the common ancestor, which consequently must in all reasonable probability have been a land plant.

Further, as the loss of seed is so tremendous, and all these plants set great numbers, it is evident that the original ancestors must have been plants which set seed freely and in great quantity, otherwise they could scarcely have adopted this mode of life. Now, as is well known, this is a character just the reverse of what is usually found in the plants of quieter water, and is therefore another argument in favour of the ancestor having been a land plant.

Again, assuming the ancestor to have been a water plant, it is evident that unless the seeds arrived at the rapids in large numbers, sufficient to allow of the enormous destruction that goes on, the chance of any modification appearing to suit the progeny to the new mode of life would be all but absolutely zero. And this is just what one cannot imagine happening. The only means of carriage to a distance that the seeds possess is to adhere to the feet of wading birds that have just been in the water, and are

walking about on the rocks afterwards with wet feet. But if the ancestral plants were water plants of quieter water, the seeds would never be in the least likely to come into contact with the wet feet of wading birds, for they would be shed into the water and not upon dry land. And they are in no way whatever suited to being shed into water. Even if it could be supposed that birds or other animals could in some way get them attached to themselves, the chances of more than an occasional one or two arriving at any given rapid would be infinitesimal, and the chances of that one successfully germinating and attaching itself would be even less.

Another fact that goes against the probability of water plants as ancestors is that the Tristichaceae and Podostemaceae could not, in their new mode of life, come into competition in any way with their ancestors, which would be living under entirely different circumstances. There does not, therefore, seem to be any reason whatever why the ancestral forms, or something fairly closely resembling them, should not still survive. Any catastrophe that so far upset the rivers as to destroy them would also probably destroy the Podostemaceae; but in actual fact there are no other water plants living in quieter water anywhere that seem to show the very slightest relationship to these families. The only family at all nearly related to them among the water plants, the Hydrostachydaceae (formerly included in Podostemaceae), is also described as living in rapid water in the mountains, *or* in estuaries,<sup>1</sup> where the water is presumably also in movement to and fro. If there ever were any water plants of quiet water allied to these families, they have entirely disappeared.

Then again, when we consider that the first adaptation of any plant, be it of land or of water, to live upon the rocks in rapid water, must have been by a single large mutation, and when we consider that a water plant of still water would in any case have to get rid of its large intercellular spaces, thus undergoing a mutation as large as that which a land plant would have to undergo, it is evident that, so to speak, we gain nothing by making the ancestor a water plant.

It is thus fairly probable that these plants could not have arrived at the rapids where they commenced as seeds of other water plants living at a distance, when we consider the seeds which they actually possess, and which must represent the seeds of their ancestors. But there is still another possibility (2 *a*) open, that they may have crept into their habitats (or arrived as seeds) from the reaches between the rapids. These reaches, however, are absolutely without any other water plants, the only things that one finds in them being land plants which have crept into the water at the edges by means of runners, &c. They are floored with moving sand, upon

<sup>1</sup> This is a very interesting point; I have little doubt that some of the Podostemaceae would also survive under such conditions. Cf. also the Algae, described by Goebel, mentioned in my Indian monograph, *Ann. Roy. Bot. Gard. Peradeniya*, vol. i, p. 420.

which water plants would find it very difficult to retain a foothold in the rapidly moving water. Reaches such as these do not, whether in temperate or tropical countries, offer satisfactory places for growth to any water plants, and it is extremely rare to find such growing in them. In any case, such plants would not be likely to have creeping roots developing secondary shoots; rather they would probably have deeply growing roots to try to get a foothold.

Assuming, however, the improbable thing that such plants did exist, it is very remarkable that they have died out and left no trace, for even if we allow Natural Selection a large part in evolution, there can be no competition between them and the Podostemaceae on the rocks, and there is now nothing in the intermediate reaches. Their habit would probably be quite different from that of the Podostemaceae, and to adopt the mode of life that characterizes the latter they would have to undergo great changes in their morphology. Land plants, on the other hand, would require a great change in their anatomy. The question is a difficult one, but it seems to me that a change in morphology, such as would likely be required here, from deeply rooting plants to creeping-rooted plants with secondary shoots upon the roots, would be a larger change than the loss of the rigid anatomy, which we know may disappear to some extent in a shoot that happens to live in water. Taking this together with the absence of any water plants in the reaches, I think we may pretty safely say that the ancestor did not live there and send seeds with the current to stick upon the rocks, while it would find it almost impossible to creep on to the rocks on account of the rush of water and sand.

We are thus reduced to the first of our suppositions, that the immediate ancestors of these plants were land plants, a supposition which from any point of view is the most reasonable. It is necessary to suppose that these plants grew on the bank of the river at the rapids, on account of the difficulty about the seeds which we have already considered, but the rapids where these plants grow are always surrounded by vegetation.

Land plants living on the edge of the stream could very well put out feelers, so to speak, in the form of adventitious roots, on which, as happens in many families, secondary shoots might arise. These shoots might very easily from the first, as is evident from the behaviour of *Littorella* and other amphibious plants, be able to go through life in water, and as the water is sufficiently aerated would never need to develop any large intercellular spaces. Thus the only adaptation that they would require for a start would be to be able to hold firmly to the rock. This could be easily enough accomplished by the development of numerous root-hairs.

The important point here is that such 'experimental' shoots might form year after year in considerable numbers without in any way endangering the existence of the parent form, until at last the necessary 'adaptive'

mutation (or mutations) appeared which enabled them to live permanently in the water. The primary shoot, developed on land, is always a last resource for survival, whereas water plants arriving from other places—or land plants either, for that matter—have to come as seeds, few and far between, and have, so to speak, nothing to fall back upon.

Having taken to the water like this, one can imagine that these secondary shoots would flourish, for they would thus at one stroke acquire a large amount of virgin territory as yet free of any plants except an occasional Moss, and at the same time would come into a medium which afforded an abundant supply of food. The seeds produced upon them would still have a fair chance of reaching the bank in reasonable numbers. On the other hand, one can imagine the primary axis to some extent handicapped by having to start the secondaries, and by the fact that their successful growth may interfere with its own, though neither of these causes is really likely to make much difference. But anyhow, it is not difficult to imagine it some day taking to the water itself, perhaps by the appearance of some mutation which might appear among the seedlings which would tend to arise upon the rocks from the seeds of the secondary shoots, or as a direct mutation, which would then become transmissible by the seeds which would be falling upon the rocks in any case. There is a difference in anatomy sometimes seen between primary and secondary shoots which may point to their having arisen at different periods in the phylogeny of the families.

It is in some such way as this, we feel sure, that these families came into existence. Water or land plants from other places than close by could never have adapted themselves to this peculiar mode of life by the aid of seeds which would only reach the rocks on rare occasions, and would then have little or no chance of retaining their position.

Be it noted that there was, so far as we can see, no absolute necessity for these ancestral plants to become Tristichaceae or Podostemaceae. They might quite well have remained amphibious. As mutation appears usually to be quite 'indefinite', giving rise to useless characters, and as the chance of a favourable mutation arriving among a chance possible assortment is almost infinitesimal, this would lead one to infer that here at least the appearance of the necessary final mutation was perhaps determined by the conditions of life, whether directly or indirectly.

In any hypothesis whatever of the origin of these plants, a very large mutation is necessary, for the change of life in beginning to live as these families do is so great that no gradual change other than one something like that which we have sketched could effect it, and even in this the last transition must be ultimately effected by a sudden mutation. The great difficulty, upon any hypothesis, is to account for the appearance of this mutation, for we know that acquired characters, such as would be the habit

of living in water for the secondary shoots which we have pictured to ourselves, are not hereditary. It is conceivable that something like memory may come in, and after they have lived like this for very long periods, may cause the necessary mutation to appear which will render the habit a permanent and practically irreversible one. In cases like *Littorella*—it is worthy of note that only genera, not families, exhibit such peculiarities—the memory may be as yet imperfect, and if one could watch them long enough, one might see a separation of a land form from a water form.

Another great difficulty which crops up upon any theory of the origin of the Tristichaceae and Podostemaceae, though it is no greater with this than with any other theory, is to explain why there are no related forms which might represent the ancestors, for as the progeny would not come into competition with the ancestors, there seems no reason why these should have disappeared. It is possible that the ancestor was already a single isolated species, and even after having given rise to the early Podostemaceae continued to live mainly in the water by secondary shoots, and was defeated by the better adapted Tristichaceae or Podostemaceae. Or again, it is possible that it was killed out in some change of conditions on land which did not affect the water plants, at least not enough to destroy them also. Or again, it may be that it will yet be discovered in some out-of-the-way district of the tropics.

Be these difficulties as they may, however, there seems good reason to suppose that these peculiar orders started as land Dicotyledons, which lived by the side of some of the many rapids in a warm country, and experimented, so to speak, with secondary shoots which they sent into the water, till on some lucky day—or other longer period of time—a mutation appeared which enabled them to live their whole life in the water from the seed, and thus opened up to them the virgin territory of the rocks in the rivers and streams of the tropical and subtropical zone.

Once the necessary mutation to enable the primary axis to live from germination in the water had been performed, this mutation would survive, and we should have the first unquestionable Tristichaceae or Podostemaceae. But having got thus far, there is, as we have seen,<sup>1</sup> no action of natural selection in the evolution of the families from this common ancestor, and the formation of about thirty genera and 200 species. The conditions of life are too uniform to allow of serious action on the part of Natural Selection, and there is not enough competition among the individuals or with other forms of life.

Now in the origin of these families there are one or two other important points that come up. In the first place it is evident that the first change, which turned the ancestral form (whatever it was) into one of these plants,

<sup>1</sup> Willis: On the Lack of Adaptation in the Tristichaceae and Podostemaceae. Proc. Roy. Soc., B., lxxxvii, 1914, p. 532.

must have been a fairly large one—either the plants can live as these plants do, or they cannot. But if so, we must allow that mutations may be 'large',<sup>1</sup> and we must admit that Natural Selection, which works by small variations, could not effect the change, both of these being points for which I have contended in other papers. No amount of 'small' changes will transfer these plants from another mode of life to their present remarkable one. As I find that very few people in Europe realize the conditions under which these plants really grow, it may not be amiss to mention that the edge of such a waterfall as Stonebyres, where the water is actually pouring over, would be covered with them were the fall in Brazil. Or the shallower parts of the Strid, near Ilkley, Yorks., where there was light enough, would similarly be covered with them in Ceylon.

In a later paper, I propose to deal with the evolution of these families, proposing a theory which appears applicable to evolution in general.

<sup>1</sup> There may have been a struggle for existence among the ancestors, but only a 'large' mutation would set a plant free from that, and we have no evidence to show that desirable changes may occur in response to any need for them, and evidence as for example with the seeds of these families, to show that they do not.

## NOTES.

**ABNORMAL PHYLLOTAXY IN THE ASH.**—As the suppression of one leaf of a pair in the case of a plant with decussate phyllotaxis is a somewhat rare phenomenon, a specimen showing this and certain other abnormalities appears to be worth describing.

The specimen referred to is a shoot of the Common Ash (*Fraxinus excelsior*, L.), which Mr. T. A. Sprague kindly handed to me for examination.<sup>1</sup> The shoot had a solitary leaf at one of the nodes, and no trace was visible externally of the second leaf which would normally be present at the node. It seemed advisable, however, to study the specimen anatomically before assuming that total suppression of the second leaf had taken place.

Though the shoot showed considerable vertical displacement of some of the leaves, it appeared obvious that the phyllotaxy was fundamentally decussate, there being no difficulty in finding the nodal companion of any leaf, except in the case of the solitary leaf mentioned above.

Transverse sections through the node with the single leaf show that there is no leaf-trace for a second leaf. Also, instead of the normal broadening of the pith into oval or elliptical form as a preparation for the separation of leaf-traces for two leaves, the pith here undergoes a one-sided alteration in form, the extension of the pith taking place only on the side towards the solitary leaf.

The position of the solitary leaf is such as would be correct for one of a pair decussating with those above and below. In view, however, of the displacement of some of the leaves, a further examination of the anatomy of the shoot in relation to the phyllotaxy was made, with the following result. Where there is vertical displacement between two leaves which appear to represent a pair, the first preparation for nodal structure is normal; i. e. a practically symmetrical broadening of the pith takes place, but the succeeding stages, leading to the separation of the vascular supply of the lower of the two leaves, are reached earlier than those for the upper leaf.

The phyllotaxy of the specimen may therefore be described as decussate, modified by vertical displacement, and by the suppression of a leaf at one of the nodes. The solitary leaf is of normal size and shows no signs of a double nature. In the somewhat similar case, however, of a specimen of *Rhinanthus Crista-galli* bearing several alternate leaves described by Groom,<sup>2</sup> a double leaf was present at the point of transition from opposite to alternate arrangement, and the phenomenon is therefore explained as 'one of morphological concrescence or of physiological fusion of impulses'.

<sup>1</sup> The shoot was taken from a hedge near Chesham.

<sup>2</sup> Groom: Longitudinal Symmetry in Phanerogamia. Phil. Trans. Roy. Soc., ser. B, vol. cc, p. 106.



The shoot of the Ash has two nodes at which the leaves are strictly opposite, but elsewhere shifting has taken place, the distance between the two leaves of a pair varying from 3 mm. to 16 mm. Displacement of this kind is not uncommon in the Ash and in many other species of plants in which the leaves are typically opposite.<sup>1</sup> A further departure from the normal phyllotaxy has also been observed in the Ash in the form of a two-fifths arrangement of the leaves,<sup>2</sup> and the same abnormality is known to occur in several other plants whose leaves are normally decussate.

On some shoots of the Ash, in the winter condition, it is easy to see that there are two buds, one above the other in the axil of each leaf. In other shoots the lower bud, which is the smaller of the two, may be very small and inconspicuous, or it may be absent. Where there are two buds, the upper one is occasionally raised distinctly above the axil, e. g. 1 to 4 mm. above it. The shoot described in this note shows an exceptional case of displacement, one bud being 25 mm. (or a quarter of the length of the internode) above the node to which it belongs. In this case an additional bud has been formed, so that there are still two buds in the axil of the leaf, the lower being the smaller. At some of the nodes of a different shoot, where the upper bud is only shifted 3 to 4 mm. above the axil, a very small third bud is again present. A comparison of these cases appears to indicate a basipetal sequence of bud-formation; hence, where two buds are present, the lower should be regarded as the accessory one.<sup>3</sup>

On examining a number of young Ash trees it was observed that considerable shifting of leaves (and also of buds) is rarer where the internodes are comparatively short, but no more definite relation than this could be discovered by comparing measurements of displacements and of internodes<sup>4</sup> in the examples specially studied.

L. A. BOODLE.

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**ON THE OCCURRENCE OF VEGETATIVE PROPAGATION IN DRO-SERA.**—The subject of the present note is the occurrence of vegetative reproduction and multiplication, by budding, in two species of *Drosera*, viz. *Drosera rotundifolia*, L., and *Drosera intermedia*, Hayne. The specimens which exhibited this phenomenon had been obtained in two consecutive years from near the large pond at Wisley. At the time of their collection (July), however, they showed no sign of the budding which they later exhibited.

The plants obtained on the first visit were transferred to the cool greenhouse at the writer's own home, and were grown in saucers on *Sphagnum* without covering. In the late spring of the following year it was noticed that young plants in various stages of development were arising from the blades of the leaves. Only a single plant developed from each leaf, but in some cases nearly all the leaves of a plant bore such

<sup>1</sup> Wydler: *Flora*, 1860, p. 628; de Vries: *Pringsh. Jahrb. f. wiss. Bot.*, vol. xxiii (1892), p. 88.

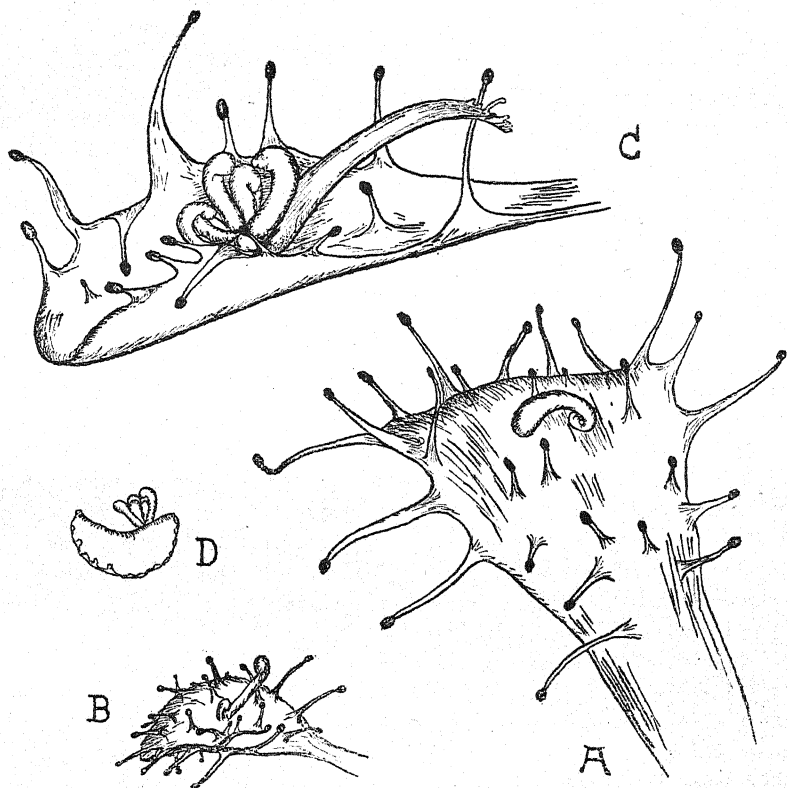
<sup>2</sup> de Vries: *loc. cit.*, p. 88; Richardson: *Gard. Chron.*, ser. 3, vol. xxxvi (1904), p. 133. Leaves in whorls of three also occur in the Ash.

<sup>3</sup> Cf. Ward, *Trees*, vol. i, p. 26. Here the upper bud is regarded as the accessory one.

<sup>4</sup> Cf. Groom, *loc. cit.*, p. 99.

buds. Most commonly it was the older leaves which were affected, but occasionally quite young leaves could be seen bearing a vigorous daughter-rosette (Fig. d).

The plants collected on the second visit were taken to the warm house at East London College and grown in pots, on *Sphagnum* as before, but in this case were covered with bell-jars. In the season following, vegetative propagation was observed on these plants also.



The first indication that a leaf of *D. intermedia* is about to produce a new plant is the appearance of a small green protuberance on the upper surface. When sectioned this is found to consist of undifferentiated parenchymatous tissue. In slightly later stages the rudiment of the first leaf develops to one side and grows very rapidly (Fig. A). The next leaf develops more slowly (Fig. B), and on the opposite side. The later leaves develop in succession, but owing to the rapidity with which the first leaf usually develops the younger ones often appear as a rosette in the axil of the oldest (cf. Fig. C). This disparity between the rates of growth of the first and following leaves was not seen in *Drosera rotundifolia*, the leaves of the daughter-plants in this case being of nearly equal size (Fig. D).

In all cases the new plants arise in close proximity to the main vein of the leaf, with which their vascular supply is at first connected. A leaf bearing a very young

daughter-plant was found to have its cells (especially those near the point of budding) completely filled with large starch grains (nearly twice the size of those normally present in the leaf-cells). Very few of the latter were, however, present in leaves bearing daughter-plants in late stages of development.

Sooner or later the leaves of the parent become detached, by the decay of their petioles, and the new individuals become established as independent plants.

Although the vegetative reproduction described has not been observed by the writer in nature, this is probably because the localities have been visited too infrequently and at unfavourable times of the year. The accumulation of starch and the detachment of the budding leaves certainly seem to suggest a definite reproductive mechanism.

E. J. SALISBURY.

EAST LONDON COLLEGE,  
January, 1915.

**ANATOMY OF THE MAGNOLIACEAE.**—During recent years the problem of the origin of the Angiosperms has attracted a good deal of attention, and views, more or less divergent, have been expressed by Lotsy, Lignier, Benson, Hallier, Senn, Newell Arber and Parkin, and others. Recent theories agree in regarding the Dicotyledons as the more primitive, but there is a want of unanimity as to the point, or points, of origin of the Monocotyledons from what is presumed to be the more ancient stock. Many hold that the Magnoliaceae are to be regarded as the most primitive of the Dicotyledons, on the ground of the resemblance between their flowers and the anthostrobilus of such types as Bennetitites. If that be the case it may be anticipated that the anatomy both of the seedling and of the mature plant will give support to the theory. Beyond a few isolated observations on some species, however, tending to show the general occurrence of secretory cells, wood fibres with bordered pits, a tendency to the formation of true vessels by scalariform perforations, and a general absence of glandular hairs, there is, so far as I am aware, a lack of any detailed comparative investigation of the anatomy of the various genera included under the order.

For some time past I have been collecting material of the Magnoliaceae and through the kindness of friends in various parts of the world I have been successful in obtaining eight out of the nine recognized genera, but as yet I have not been able to acquire specimens of *Zygogynum*. I would esteem it a favour if any one having access to this New Caledonian form would be so kind as to send me specimens. During the past winter I have investigated the adult anatomy of *Drimys Winteri* and endeavoured, though unsuccessfully, to raise seedlings from seeds supplied through the courtesy of several correspondents.

The literature on the subject is not very extensive, and beyond the papers recorded by Solereder (Syst. Anat. Dicot.), few investigations have been published on the subject. Strasburger, working on *Drimys*, draws attention to the gymnospermic character of the xylem coupled with the angiospermic mode of development of the megaspore, and Maneval has also studied the embryonic structure in *Magnolia* and *Liriodendron* (Bot. Gaz., vol. lvii). Paul Parmentier (Bull. Scient. de la France et

de la Belgique, t. xxvii, 1895) claims to have seen true vessels in two species of *Drimys*, viz. *D. Mülleri* and *D. vascularis*, but it may be questioned whether these forms ought to be included under the genus.

An examination of the anatomical structure of *Drimys Winteri* and *Drimys odorata* has established the following points:—

1. Vessels (tracheae) are absent.
2. Bordered pits with X-like apertures similar to those in *Cycas* and some Conifers are present.
3. No longitudinal parenchymatous strands occur in the wood.
4. The sieve-tubes have bevelled ends, bearing several plates like those found among Pteridophyta, and appear to be accompanied by companion cells.
5. A broad transitional region exists in the wood of young twigs, from the spirally thickened protoxylem elements to the tracheides with bordered pits of the mature wood.
6. Resin, or a secretion of a resinous nature, occurs in large quantities in the parenchyma, and drains into resin 'ducts' which are of the nature of simple intercellular spaces.
7. The medullary ray cells are pitted and elongated in a vertical direction, and in a tangential section the rays are multiseriate in the middle, becoming uniseriate above and below.

These anatomical points, among others, recall pteridospermic and gymnospermic peculiarities of structure. Thus the bevelled-ended sieve-tubes with lateral plates and the absence of vessels are characteristic of Ferns and their allies. The bordered pits agree in structure with those of most Conifers and Cycads, although it should be remembered that *Marsilia* also possesses tracheides with similar bordered pits. The absence of longitudinal strands of parenchyma in the mature wood may be a primitive or a reduced character, but this point can be decided only by comparison with other genera.

This preliminary note is intended only to indicate the general lines on which the present investigation is being carried out, with the hope that Systematists interested in this branch of phylogeny may be induced to aid me either by sending material or by gift of 'separata' of their writings on the subject.

MARGERY KNIGHT.

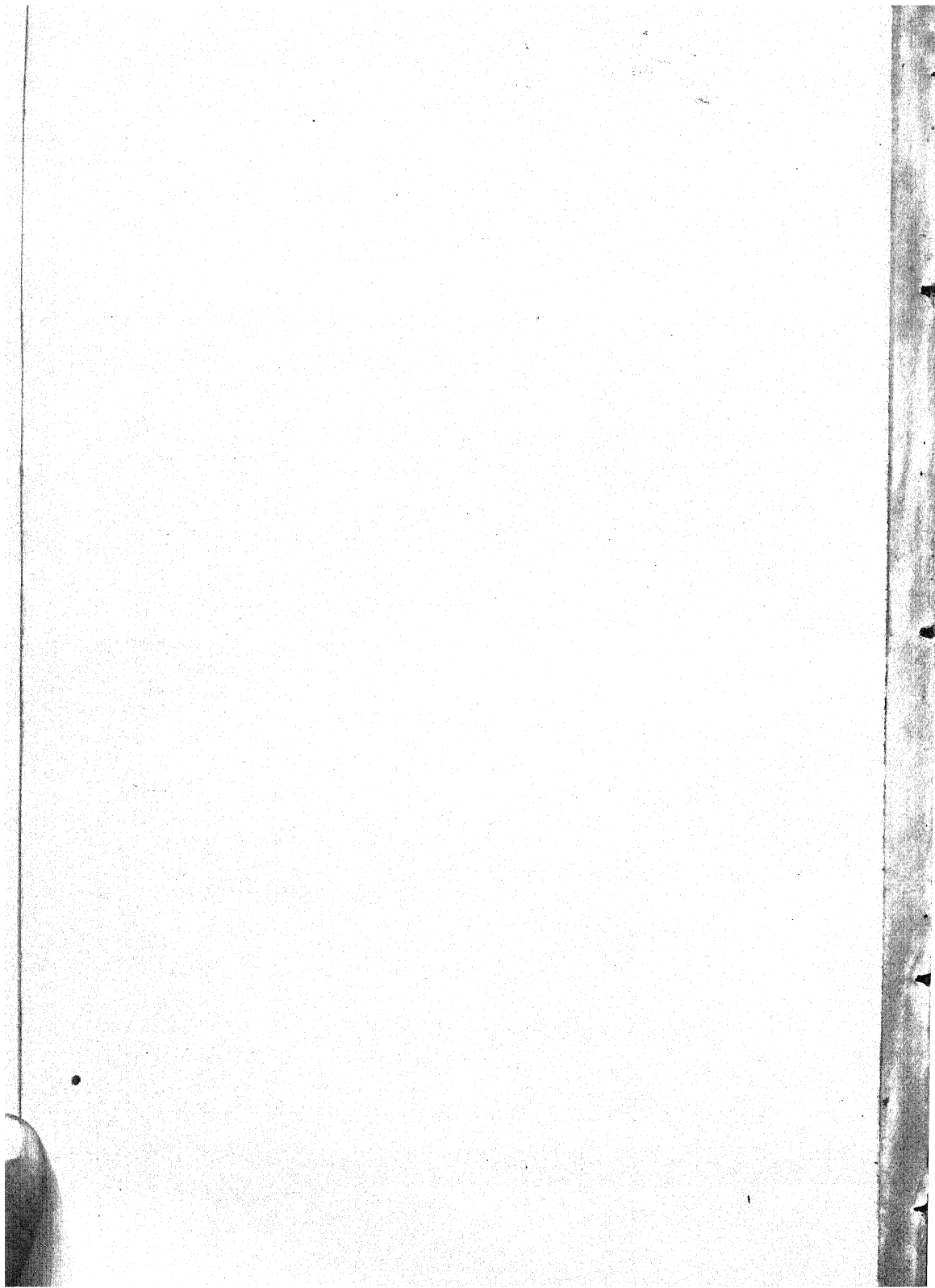
HARTLEY BOTANICAL LABORATORY,  
UNIVERSITY OF LIVERPOOL.

#### CORRECTION.

A. S. MARSH: The Anatomy of some Xerophilous Species of Cheilanthes and Pellaea, vol. xxviii, 1914, p. 627.

lines 5 and 7, for Santa Catalina, California, read Santa Catalina Mountains, Arizona.

line 6, for California, read Colorado.



# Studies in the Physiology of Parasitism.<sup>1</sup>

## I. The Action of *Botrytis cinerea*.

BY

WILLIAM BROWN, M.A., B.Sc.

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### A. INTRODUCTION.

THE physiological relation of host and parasite is a subject urgently in need of investigation at the present time. On a knowledge of such relations must depend any further insight into the nature of immunity and susceptibility of plants to disease. In the case of the more highly specialized parasites there is considerable difficulty in studying their physiological relations to their host owing to the complexity of the relationship, which often amounts almost to symbiosis, and to the difficulty of growing the parasites independently and of bringing about infection at all times of the year. It seemed probable, then, that a closer investigation, by modern biochemical methods, of the mode of action and method of infection of some of the simpler parasites which can be easily cultivated would be of great value. A knowledge of the relationship of such forms to their hosts should lead the way to a better understanding of the more highly specialized parasites. It was with this view that the present investigation was undertaken.

<sup>1</sup> This is the first of a series of studies which are being carried out in the department of Plant Physiology and Pathology of the Imperial College of Science and Technology.

*Botrytis cinerea* is a member of the large physiological group of Fungi known as 'Facultative Parasites', the members of which while normally saprophytic are able under certain conditions to live parasitically. Among Fungi of this type, the subject of the present investigation is somewhat pre-eminent, partly on account of its ubiquity, partly by reason of the striking features of its parasitic attack. When to these is added the fact of its being very amenable to cultural treatment, it is not surprising that this form has become a classical one for the study of the physiological questions relative to this type of parasitism. As a result of these researches certain features of this type of parasitic activity may now be considered as being firmly established. Prominent among these is the one due to de Bary (1), and since his day frequently confirmed, that such Fungi possess the power of killing and disintegrating the tissues of the host in advance of their growth, so that properly speaking they are not parasitic upon the living host at all, but live merely on its dead remains. This phenomenon of 'action in advance' de Bary set down to the excretion by the tips of the advancing hyphae of some soluble substance which is capable of bringing about the observed changes—changes consisting, firstly, in the solution of the cell-wall, or at least of certain of its constituents, so that the tissue loses its coherence; and, secondly, in the killing of the living protoplasmic contents themselves. So far we find general agreement, but when we come to treat of the nature of this soluble substance, of what it consists, of how many chemical entities it consists, and what share in the process is assignable to each, we meet with considerable divergence of opinion. This will be made clear from a review of the literature dealing with this question.

#### B. HISTORICAL.

De Bary (1), in a paper now classical, investigated the parasitism of *Sclerotinia Libertiana*, a form closely allied morphologically to *Botrytis cinerea*, and showing many similarities in its mode of parasitism. By using the expressed juice of certain plant organs (roots of carrot, &c.) which had been infected and overrun by the fungus, as also the liquid exuded in droplets from the young sclerotia of the fungus itself, he was able to show that the active principle was thermolabile, and so concluded that it was of the nature of a ferment. The cell-wall dissolving activity of the fungus he unhesitatingly ascribed to this enzyme; as to the toxic activity he was somewhat doubtful. He discussed the possibility of the latter being due to some soluble oxalate, and while showing that oxalates do occur in certain secretions of the fungus, he was unable on quantitative grounds to ascribe to these the markedly destructive effects of the fungus. His final conclusions may be taken as summarized in the following quotation: 'The difference (between boiled and unboiled extract) is indeed a quantitative one as far as can be observed. . . . With the liquid from sclerotia the same

differences appear, though less prominently—the boiled liquid has here a relatively greater effect.'

Marshall Ward (2), in his treatment of this problem, made flask cultures of *Botrytis* on prune juice. After the lapse of three weeks, he removed the superficial web of mycelium, which was then washed, dried, ground to a powder, and extracted in water. He was able to confirm de Bary as regards the presence of a cell-wall dissolving enzyme; as regards the toxic nature of the extract he was unable to make any advance.

Precisely similar results had been obtained a short time previously by Kissling (3), the same type of method being employed.

Nordhausen (4) strongly emphasized the twofold aspect of the phenomenon, enzymic and toxic. The following represents his conclusions: the active substances are 'apparently in part likewise enzymes, a conclusion which I reach from experiments similar to those of de Bary, although it is not excluded that oxalic acid under certain circumstances plays a part. That the latter, however, is not alone responsible for the toxic action on plant tissue is shown by the case of *Aspergillus niger*, which secretes oxalic acid in large quantities, even more so than *Botrytis*, without showing in any similar degree the marked parasitism of the latter.'

Behrens (5) used the expressed juice of fruits which had been infected with certain Fungi; thus in one case that of a pear infected with *Mucor stolonifer*, in another that of an apple infected with *Penicillium luteum*. In the latter case he mentions that the culture was allowed to develop for three months before the extract was investigated. These juices he found to be toxic to the mesocarp cells of *Symphoricarpos* berries, nor were the toxic effects reduced by boiling. Hence he concluded that the toxic substance was neither of volatile nor of enzymatic nature.

Smith (6) compared the actions of weak oxalic acid solutions (0.01 per cent. to 1 per cent.) and of a mycelial extract of *Botrytis* on certain plant tissues (stem of lettuce), and while noting that the effects were not similar in all particulars—especially in respect of the post-mortem reactions induced—he concluded that oxalic acid, even in the lowest concentrations employed, was sufficient to produce the changes observed. He even goes so far as to state, on the basis of certain maceration effects of the acid, that the cell-wall dissolving effects of the fungus may be set down to this acid, quoting from de Bary that so much as 0.3 per cent. oxalic acid may occur in mycelial extracts. This author thus occupies an extreme position among those who ascribe an agency in this question to oxalic acid.

As papers cognate to this subject may be cited those of Jones (7), Potter (8), and Van Hall (9). These investigations refer to bacterial plant diseases, and in each case it was shown that a cell-wall dissolving ferment could be obtained from media in which the organisms had been cultivated. This ferment is no doubt very similar to the *Botrytis* ferment under



consideration. On the question as to the presence of the toxin these authors were unable to make any notable contribution.

*Criticism.* Viewed generally, the methods of preparing the fungal extract which have been adopted by previous workers fall under the two heads:

1. *Extraction from the old mycelium.* This is the method of de Bary, Marshall Ward, Kissling, Smith, and Nordhausen.

With respect to this method it can reasonably be objected that the secretions of an old mycelium do not necessarily bear any close relationship to those of a young vigorous culture. It is to be borne in mind that the active invading portion of the fungus is essentially of the nature of a young and fresh culture. In order therefore to study the nature of the active principle of the fungus, it is necessary to examine the secretions of young and vigorously growing hyphae. In this way only can the complications due to the presence of waste products ('staling' products) be avoided.

2. *Extraction from plant organs* (fruits, tubers, &c.) which have been overrun by the fungus. This method was employed by de Bary, and more particularly by Behrens.

The time required for the complete invasion of a compact structure such as a good-sized fruit may be considerable; in one case, for instance, Behrens employed a three months' old culture of *Penicillium luteum* on apple. It will thus be seen that the objections relative to old and (in the main) stale cultures put forward under the previous heading apply here with equal force. Furthermore, this method presents additional complications. In extracts of this sort there are present substances derived both from the fungus and from the host, in varying amount according to the degree of invasion at the time of extraction. Now when we bear in mind the plasmolysing effect of plant and especially of fruit juices, and furthermore that plasmolysis normally induces death of living tissue, it is easy to see that such extracts as those of de Bary and Behrens can give no certain indication of the primary toxic principle concerned. It will be shown later that the plasmolysing effect of a plant juice is greatly reduced by the growth in it of a fungus. Nevertheless the presence of the unknown remainder constitutes in all cases a complication which certainly should be avoided.

A similar criticism would also apply to the bacteriological investigations above mentioned. Here, however, the active solutions obtained were so weak that little was attempted in the way of studying the question of toxicity.

The work of Smith calls for special criticism. His method was to compare the action of mycelial extract with that of various strengths of oxalic acid, and from certain similarities he concluded that this acid played a great part in the changes concerned. Other acids he found behaved

in this respect like oxalic acid; he chose the latter from its known occurrence in the fungus. In one important particular, however, he is at fault, inasmuch as the starting-point of his study is a strange misquotation from de Bary. The latter he cites for the statement that oxalic acid of 0.3 per cent. strength may be found in extracts of *Sclerotinia*, whereas de Bary states categorically that he was unable to demonstrate any *free* oxalic acid, but that this substance occurs solely in the form of its salts (potassium or calcium). Smith himself records 2 per cent. oxalic acid in extracts of old mycelia, but here he is undoubtedly including oxalates, both soluble and insoluble. In the low concentrations employed by Smith, potassium oxalate has only a very slow toxic action, and its macerating action is nil at all concentrations. Such being the case, it cannot be allowed that Smith's contributions on this particular question are really helpful.

Apart from these considerations, a perusal of the literature convinces one that hitherto no one has succeeded in obtaining a really strong solution of the active principle of *Botrytis cinerea* or of any of its near allies. This we may conclude from the times required to bring about the various decompositions as recorded by the different investigators. Thus de Bary and Marshall Ward, in testing the action of their extracts on thin sections of plant tissue under the microscope, speak of a definite change being observable after some hours, while in the case of Behrens the action of the extract was allowed in some cases to proceed for twenty-four hours before observation was made. Now as regards the toxic action of an extract, it is absolutely imperative that the transformations be carried out within a comparatively short time. In work of this description—where the behaviour of living tissue is used as the indicator of a reaction—the complete exclusion of micro-organisms is a practical impossibility, and therefore observation can only be extended with confidence over the period during which bacterial activity is inconsiderable and while there is no accumulation of bacterial excretory products. How long this period may be will depend on various factors, prominent among which is the temperature maintained; but, speaking generally, it has been found that under ordinary laboratory conditions it is only with the help of special control measures that observation can be safely extended up to and beyond twenty-four hours from the commencement of the reaction. It is possible to extend the period of observation by lowering the temperature, a procedure which greatly retards the progress of bacterial contamination, without retarding in anything like the same degree the activity of the extract. Needless to say the employment of antiseptics is not permissible in studies of the toxic action of an extract.

The aim, then, of the present investigation was in the first place to prepare an extract from young hyphae alone, and it was hoped that this extract might prove to be of a sufficiently powerful nature to

recommend it for use in such an investigation as it was intended to prosecute. This anticipation has not been disappointed. It was found possible to obtain from young recently germinated spores of *Botrytis* an extract much more powerful than any hitherto attained, and furthermore a method has been elaborated for obtaining such material in fairly large quantities without prohibitive labour. As the method, though containing nothing new in principle, is novel, inasmuch as no such procedure has hitherto been attempted, and as it may be found to be applicable to other studies along similar lines, it will be described in some detail.

### C. PREPARATION OF STANDARD EXTRACT.

As it was thought advisable to use the same strain of fungus throughout, certain cultural precautions were taken in order to retain its vigour, and more especially its capacity for spore production. Up to the present time two primary cultures of *Botrytis cinerea* have been employed, one in the earlier stages, the other throughout the remainder and greater part of the investigation.<sup>1</sup> The former strain ceased after a few months to produce spores in abundance, and no treatment, such as alteration of temperature or of medium, was effective in restoring it to its original freely sporing condition. Up to this stage the practice had been to reculture the stock cultures rather frequently—once a fortnight—and to incubate them at 25° C. With the new culture this procedure was modified, stock cultures being kept at laboratory temperature (about 20° C.), and only undergoing reculture at much longer intervals—actually once in about three months. The present strain has now been in culture in this laboratory for a year and a half, in which time it has reached its sixth generation, and it still spores as freely as ever. Stock cultures are made in large ‘boiling tubes’ on the potato agar medium to be described below.

The first object is to provide an abundant supply of spores. This is done by the usual Petri-dish method, the culture medium being inoculated with the spores prior to pouring in order to obtain uniformity of development over the plate. As culture media, trials were made of turnip, prune, and potato agars. From the point of view of copious spore formation, the last is incomparably the best, and it has accordingly been employed throughout. It has the following composition:

|               |           |             |
|---------------|-----------|-------------|
| Peeled potato | . . . . . | 200 grs.    |
| Agar          | . . . . . | 10 grs.     |
| Water         | . . . . . | to 1 litre. |

The potatoes are boiled in water till they form a mush, which after cooling is pressed through a muslin bag in order to break down the larger

<sup>1</sup> Both cultures were obtained from the ‘Centralstelle für Pilzkulturen’, Amsterdam.

lumps. The subsequent treatment is as usual. Plate cultures are incubated at 20° C. (at 15° the spore yield is very much diminished). Spore formation begins in about 4 days; in 10 days to a fortnight the plates are ready for the subsequent treatment.

The problem now is to obtain the spores from the plate cultures. For this purpose each plate is covered with a layer of distilled water; then by gentle rubbing with the finger, beginning at the centre and working to the margin, it is possible to expel the entangled air without at the same time unduly contaminating the atmosphere of the room with spores, though this is unavoidable to some extent. The whole aerial portion of the culture—mycelium and spores—is now rubbed off by gentle scraping with a blunt knife. With a little practice it is quite easy to perform this operation without disturbing the underlying solid medium, so that, apart from the fungus itself, only liquid substances are removed from the plate. The fungal debris, &c., is now filtered through a fine clean muslin cloth (20 threads to the cm.). The spores and finer particles pass through, whereas the general mycelium, apart from occasional very short pieces of hyphae, is completely held back. The spore suspension is now centrifuged at a moderate speed. By this means it is possible to separate the heavy spores which are readily thrown down from the finer debris which remains in suspension. The 'wet' volume of the centrifuged spores is noted for purposes of the following treatment.

The centrifuged spores thus obtained in a practically pure form are now suspended in definite proportion in a nutrient liquid and spread over a glass plate in order to germinate. The following general considerations may be noted:

#### *1. Length of Period allowed for Germination.*

As it was proposed to examine the physiological conditions prevailing within the 'infection drop'<sup>1</sup> with the object of throwing some light on the manner in which the fungus first actually enters the host plant, an attempt was made to obtain an extract from the fungus at a stage comparable with that at which it actually penetrates the host. The period elapsing from sowing to penetration varies somewhat with different hosts and under different conditions, but generally speaking it may be set down as lying between 12 and 24 hours. Throughout this work the spores were allowed as nearly as might be 23 hours' germination, this being considered sufficiently close to the ideal time, as well as offering conveniences for the systematic day-by-day repetition of the process which the method entails.

<sup>1</sup> By this is meant the drop of fluid in which sowings of the fungal spores are made on the surface of the host plant. With Fungi of this type, this represents the usual procedure in artificial infection.

*2. Uniformity and Completeness of Germination.*

For this purpose, the factors of importance are—

- (a) The spores must be uniformly distributed throughout the nutrient fluid ;
- (b) The quantity of spores per unit volume of nutrient should not exceed a certain limit ;
- (c) The film of nutrient on the plate should be of uniform depth throughout.

The details of the method, as given below, were only gradually evolved, and being based on numerous subsidiary experiments may be taken as representing the best set of conditions from the point of view on the one hand of effective and uniform germination, and on the other of economy of space and labour.

The method of dealing with 0.5 c.c. of centrifuged spores, which is about the amount obtainable from two, fairly good, 8 cm. diam., Petri dish cultures, may be stated. This quantity of spores is suspended in 50 c.c. of the nutrient fluid. The plates on which the spore suspension is sown are flat, circular, of 8 in. diameter. Each plate is supported on three wedge-shaped corks in the bottom of a large Petri dish, the atmosphere of the latter being kept moist by the usual devices. Each plate is accurately levelled by means of a spirit-level previous to sowing, this being effected by manipulation of the supporting corks. Immediately before each plate is sown, the spore suspension must be agitated. This can readily be effected by blowing in air through the pipette which is employed for measuring out the allowance for each plate. The spore suspension is now spread over the plates up to  $\frac{1}{4}$  in. from the margin at the rate of 5 c.c. to each, spreading being most readily effected by means of the finger. With practice it is quite easy to sow as many as 20 plates in half an hour. The spores are now left to germinate at the ordinary laboratory temperature (about 20° C.).

With the plates employed and with the given suspension density of spores (0.1 c.c. spores to 10 c.c. nutrient) it has been found that any reduction from the amount given above (viz. 5 c.c.) for each plate has resulted in a diminution of yield together with increased tendency to lack of uniformity in germination. Any reduction in the amount of liquid should be accompanied by a corresponding reduction in the density of suspension.

In the earlier stages of this investigation, the nutrient fluid employed was a commercial grape preparation (*Welch's Grape Juice*), and for purposes of the manufacture of spore material this medium proved quite satisfactory. This preparation, however, when laid in the form of drops on the surface of plant tissues (leaves, &c.) has a very strong plasmolysing and killing effect ; and in view of the fact that one object of the research was to

institute a comparison between the action of the extract obtainable from the germinating fungus and that of the fungus itself upon the living tissue, this feature was very objectionable, and an effort was therefore made to find a substitute. This was found in Turnip extract, which has proved to possess many advantages. Besides considerations of cheapness and availability, it is superior to 'Grape Juice' in its much slighter plasmolytic effects while equalling it in the quantity, and more than equalling it in the quality, of fungal material which it furnishes. It also possesses the considerable advantages that it is only slightly coloured, and its stock solutions can withstand repeated heatings without deterioration, whereas 'Grape Juice' suffers a definite loss by precipitation at each sterilization. This turnip medium is prepared as strong as possible—that is, the turnips (white) are autoclaved without addition of water, and the juice subsequently extracted under pressure. The most suitable medium is derivable from solid half-grown turnips, that from old vacuolated ones being considerably weaker and affording a smaller yield of germinated spore material.

The germination phenomena need not be described in detail. After four hours, commencement of germination can be seen in a small number of spores; after eight hours, germination is very general, but is variable in amount, the germ tube ranging from a mere papilla to a tube of four spore-lengths; at the end of the period allotted, apart from occasional spores which have remained clumped together and which may show various stages of arrested development, germination is very generally well advanced. The young hyphae, by reason of the comparatively thick rate of sowing adopted, are all closely intertwined and their individual appearances can only be readily seen by tearing out a small portion of the 'weft'. The average length of hypha depends naturally on the strength of the particular turnip extract employed, but it may be taken as approximating to 20-40 spore lengths. While each spore normally puts out a single germ tube, bipolar germination is frequent, in which case the length of each tube is less than in the normal case. Side branches of first order are not at all uncommon.

At the conclusion of the period of germination the plates thus present a continuous weft of interlocking hyphae, and the whole film may be removed as such, much in the same way as a gelatine film may be removed from a photographic plate after immersion in warm water. The fungous film offers usually considerable resistance to removal, being firmly attached to the plate, a phenomenon which finds its explanation in the very general development of incipient attachment organs. This is readily verified microscopically, a large proportion of the hyphae showing at their tips the flattened slightly swollen appearance characteristic of the early stages of these organs. The formation of these attachment organs is very strongly marked in sowings in Turnip extract, more so than for instance in sowings in 'Grape Juice', and it is at any rate plausible that the superior quality of

the material obtainable by using the former liquid is in some way related to this marked development of attachment organs.

In practice the removal of the film from the plate is most readily effected by means of a glass slide. The spore material is now thrown on to a muslin cloth, the edges of which are wired to a tripod in such a way as to form a bag, and the whole mass is subjected to vigorous washing, with stirring, under the tap. Any ungerminated spores, minor débris as well as the nutrient medium, are by this means washed away, while the wet-like nature of the mass of germinated spores prevents their passing through. This washing is continued for ten to fifteen minutes, and is followed by three successive washings in a large quantity (half a litre) of distilled water. The spore material is now strained as far as possible, spread evenly over a glass plate, and dried over calcium chloride *in vacuo*. The fungus material when dry is scraped off and ground in a mortar with clean, dry quartz sand. Throughout this work equal weights of fungus and sand have been used. If perfectly dry—i. e. immediately after removal from the desiccator after overnight exposure to the calcium chloride—the fungal skin is brittle and lends itself quite readily to grinding. When kept for some time in the ordinary laboratory atmosphere, being hygroscopic, it gains slightly in weight and in this state is tough and difficult to reduce to powder. In practice an attempt was made to make the grinding of successive lots as far as possible uniform in degree, this being done by the use of a system in grinding. The ground powder is a uniform grey, and the degree of grinding can be estimated roughly by the change in colour from the black of the unground to the light grey of the well-ground spores. Grinding has throughout been performed by hand, though some sort of mechanical apparatus could no doubt be readily fitted up. In all cases the amount of grinding has not been stinted, and examinations of a little of the wetted powder which have been made from time to time under the microscope have shown that only occasional spores escape destruction; recognizable traces of hyphae are also rare.

As the materials of different days' growth vary to a slight extent in the activity of the extract to which they gave rise, the practice has been to collect a considerable quantity of material, which is then intimately mixed up before being used for experimental purposes. This method is justifiable on the ground that, as far as can be seen, the dry powder preserves its activity undiminished for a very considerable time. Thus in one instance a certain tube of material appeared to possess undiminished activity after a two months' interval.<sup>1</sup>

<sup>1</sup> This is in accordance with the behaviour of other similar substances in the dry form. In the present case an exact proof is impossible on account of the difficulty of obtaining identical substrata at different times. The above conclusion is based upon the general effectiveness of the material upon a variety of substrata.

The spore material is extracted by suspension in distilled water, care being taken to keep the débris in suspension by shaking at intervals. By subsidiary experiments (using the quantitative method to be described below) it was shown that by increasing the amount of powder suspended in a given volume of water, the activity of the resultant extract increased, but only up to a certain point, further increases in the proportion of powder giving no appreciable increase in the activity of the extract. This limiting activity of extract is obtained when extraction is made in the proportion of 0.2 gr. powder (= 0.1 gr. fungus + 0.1 gr. sand) to 3–4 c.c. water. Thus the difference between the activity of, say, a 0.2 gr. in 2 c.c. and a 0.2 gr. in 3 c.c. suspension is, with powder of normal quality, scarcely demonstrable. In view of these considerations, the spore powder has been extracted throughout in the proportion of 0.2 gr. to 3 c.c. water, the full activity for the powder being thus obtained.

In view of the statement of Michaelis (Abderhalden's *Handbuch d. biochem. Arbeitsmethoden* III, i, p. 13) that in such cases, where the enzyme or similar substance is contained on the surface of an insoluble powder, an extraction of twenty-four hours' duration is required in order to ensure uniformity of extract in different experiments, a series of experiments was set up to determine what time of extraction is necessary in order that the extract may reach its limiting strength. Extractions were made for periods of  $\frac{1}{2}$ ,  $\frac{1}{3}$ ,  $\frac{2}{3}$ , and 1 hour, the process being stopped at the end of each period by centrifuging off the débris. These extracts were compared by the quantitative method to be described later. In the case of the  $\frac{1}{2}$ -hour extract only was there any indication that the full strength had not been reached. The three other extracts were of equal strength within the limits of experimental error. Throughout this work, one hour's extraction has been allowed, this time being chosen as representing safety as well as offering conveniences for the carrying out of the other experimental details involved. The liquid is finally cleared by centrifuging for three minutes at the highest speed available (3,000 revs. per minute), decanted, and again centrifuged and decanted. The liquid thus obtained is the crude extract which has been employed in the present investigation. It possesses a pale straw-colour, is opalescent, and has a characteristic 'mouldy' smell.

Though the experimental method as sketched above seems somewhat laborious, it is by no means unduly so, and it is quite practicable to prepare in this way quantities of material comparable to those which are usually employed in enzymic studies. The following figures will suffice to show how the method works out, representing as they do a fair average:

From two Petri dishes of 8 cm. diameter are obtained 0.5 c.c. spores (wet volume); the latter are sown in 50 c.c. Turnip extract on 10 plates. The weight of germinated material when dried = 0.7 to 0.8 gr. Thus the powder obtained = 1.4 to 1.6 gr. For the preparation of standard extract,



this quantity of material is suspended in 21 to 24 c.c. water, furnishing after centrifuging 18 to 20 c.c. of extract. As the result of 26 days' preparation of material, a maximum of 10 plates being employed, a quantity of 35 gr. of powder was collected. This represents a quantity of about 420 c.c. of standard extract. Again, as the result of 10 days' preparation, about 25 plates being sown daily, a stock of upwards of 60 gr. of powder was gathered, representing about 750 c.c. of extract. It is thus plain that the experimental treatment of this subject, according to the routine here adopted, offers no greater difficulties from the point of view of the availability of material than are met with in enzymic studies generally.

In carrying out the above-described routine, no special precautions against bacterial contamination have been found necessary. Bacterial action would be considered most likely to show itself on the germination plates at the end of the germination period. No doubt bacteria do occur, though several examinations with the microscope have failed to demonstrate their presence. This state of affairs is probably to be set down partly to the acidity of the turnip medium, and partly to the vigorous development of the fungus spores. In any case a limited bacterial development is of no importance, as both bacteria and bacterial products are washed away in the further treatment of the fungal material.

#### D. QUANTITATIVE METHOD OF STUDYING ACTION OF FUNGAL EXTRACT.

The basis of the method is the capacity of the fungal extract to destroy the coherence of any susceptible tissue which is placed in it for a sufficiently long time. The method is as follows:

From a tuber or other fleshy organ, an axial cylinder of about  $1-1\frac{1}{2}$  cm. diameter is cut by means of a cork-borer; this is cut across in the middle, and from the surfaces so exposed transverse sections of  $\frac{1}{2}$  mm. thickness are cut by means of a hand microtome.<sup>1</sup> The discs are now freed from contained air by injection under the pump with water. After thorough washing in distilled water, they are placed in the extract and the time noted that is required to bring about 'loss of coherence'. For purposes of the present quantitative test, *coherence is said to be lost, when the discs as tested by hand offer no perceptible resistance to a pulling stress.*<sup>2</sup> The time from commencement of action to 'loss of coherence' gives a measure (inverse) of the activity of the extract.

<sup>1</sup> Leitz, Wetzlar.

<sup>2</sup> This stage does not represent the end-point of the macerating action even in its macroscopical aspects. The discs later become so fragile that it is impossible to handle them without rupture, though they do not actually fall asunder of their own accord. This stage, however, is difficult to determine, as there is no means of regulating or measuring the small strains which bring about rupture.

The limitations of this method, and the accuracy of which it is capable, are discussed under the following five headings:

(i) *Nature of tissue employed.* It is obvious that a tissue suitable for this purpose is one which in its fresh state possesses a marked degree of coherence. Such a tissue, as for instance that of the pear or even of many varieties of apple, is of little value; on the other hand, turnip and potato tissues are eminently adapted to the present purpose, and these have accordingly been generally employed.

The ideal tissue is one which allows of discs being cut which are uniform in quality throughout. Ordinary leaf tissue cannot therefore be employed, and in practice we are confined to the use of fleshy tissues. Even with these it is in general impossible to prepare discs which are quite uniform throughout. Thus even in the centre of the potato small vascular strands occur which produce local lack of uniformity. In cases where these are abundant, the tissue should be rejected, and in all cases care must be taken that these small vascular strands do not run in the plane of the section and more especially in the direction in which coherence is tested. A tissue suitable for the present purpose may therefore be defined as one furnishing discs which on microscopical examination are seen to consist of a ground-mass of uniform parenchyma cells with a limited number of islands of vascular (more resistant) tissue.

(ii) *Accuracy in thickness of the sections employed.* The degree of accuracy obtainable in the disc-cutting process depends to a considerable extent upon the nature and physiological condition of the particular tissue. It also depends upon the thinness of the discs cut, there being a limit of thinness beyond which the microtome ceases to perform satisfactorily in this respect. Care must be taken that the cylinder of tissue is sufficiently rigid not to yield to the knife. This consideration requires that the cylinder, in addition to being of a certain stoutness, should be quite turgid, a condition which is readily obtained by injection with water previous to cutting. In the case of potato tissue, the upper limit of variation in thickness of the microtomed discs was determined as 4 per cent.<sup>1</sup>

(iii) *The determination of the 'end-point' of reaction.* It must be conceded that the arbitrarily chosen end-point does not represent any definite stage of the reaction. It is simply that stage at which

<sup>1</sup> This was measured by cutting a series of sections, which were then washed, rapidly dried between filter-papers, and weighed. The following were the figures obtained:

|  |            |
|--|------------|
| Max. weighing . . . . .                                | 0.088 gr.  |
| Min. „ . . . . .                                       | 0.085 gr.  |
| Average weight of sixteen discs . . . . .              | 0.0867 gr. |
| Max. variation from mean . . . . .                     | 0.0017 gr. |
| Max. variation among the individual readings . . . . . | 0.003 gr.  |

This variation of 0.003 gr. in 0.0867 gr. is approximately 4 per cent.

coherence has been so far reduced as to be imperceptible when tested by the hand—that is, when the disc is pulled from opposite sides it separates without perceptible resistance. The exact position of this point in the series of changes in the tissue brought about by the extract obviously depends on the ‘stimulus threshold’ for resistance to pull of the particular observer. This limiting value varies with the same observer from time to time, and thus a varying personal equation is introduced. In cases where two extracts of approximately equal strength are being tested, it is obvious that if the determinations are made within a short time of each other, the personal variation is small. When two extracts of widely different activities are being compared, it may happen that the end-points are reached at widely different times, in which case the personal factor may be different in the two determinations. It is, however, plain that in such a case the necessity for accurately determining the end-point is diminished.

The accuracy of determination of the end-point obtainable in practice may be gauged from the following figures. In the case of an experiment which would be set down as finished in fifty minutes, it is quite possible to convince oneself that a definite degree of coherence is perceptible after forty-five minutes; in other words, it is quite feasible in an action lasting fifty minutes to determine the end-point to within five minutes. This gives an error limit of about 10 per cent. Furthermore, when discs from the same region of an approximately uniform tissue are tested with the same extract, their end-points are found to show agreement to within 10 per cent. This figure then represents roughly the limit of variation which ceases to be significant. In the following it will be seen that the differences of activity noted are as a rule much greater than this, and no conclusions are drawn from observed differences in activity in which it was not perfectly certain that the differences observed lay far beyond the limits of this observational error of 10 per cent.

(iv) *The varying nature of the actual substrate.* There is wide variation in sensitiveness of the different potatoes, turnips, &c., employed. Thus with the same extract a variation of 100 per cent. has been observed in different potatoes. The same has been shown to apply to turnips. This consideration renders the comparison of results of different series of experiments difficult, and in practice this was carried out by comparing each with a standard. This is furnished by the standard extract above described, a common stock of spore material being kept for purposes of this day-by-day comparison. When, however, the comparison of two sets of experiments was considered to be of critical importance, it was always possible to carry out the observations side by side on the same substrate.<sup>1</sup>

<sup>1</sup> It might be thought feasible to cut from the same potato a large number of discs, which could then be kept in presence of an antiseptic and used for standardizing purposes. This method

(v) *The stability of the extract.* The extract is not a stable solution, but loses its activity with lapse of time. After twenty-four hours in presence of chloroform, it has lost slightly in activity; after a week its activity is very small. The effect of this deactivation with time is to prolong the slow reactions unduly. This error can of course be corrected by renewal of the liquid from time to time. In the average experiment, lasting less than six hours, this procedure is not necessary, and the error introduced is not appreciable.

*Other Quantitative Methods.* A variety of other methods has been tried from the point of view of a quantitative treatment of the subject. As these have been comparative failures, it is only proposed to mention them briefly.

A substrate was prepared for the enzyme in the following manner. Turnip tissue was pulped, freed by prolonged washing from soluble materials, dried and ground to a powder. This may be considered to be in the main a cellulose powder. Water suspensions of this powder were added to the extract, and the action was estimated after a time by Fehling's solution. This experiment was controlled by a similar one in which deactivated (see later) fungus extract was employed. The conclusion reached was that while the figures lay in the right direction, they were far too small to encourage the hope that the method might be serviceable. Quite apart from being very laborious, the method appeared infinitely inferior to the one adopted. The same remarks apply to a number of experiments in which an attempt was made to use as substrate a 'calcium pectate' extract of turnip tissue, prepared according to the method described by Behrens (l. c.).

In another series of experiments the killing action of the extract was followed by measuring the rate of escape of certain constituents of the tissue. Experiments along these lines were tried with tissues of turnip, onion, and beet. In the first two of these the action was estimated by Fehling's solution, in the last it was measured colorimetrically. The conclusion reached was that this represented a possible method which, given a suitable tissue, might furnish results of value. It did not appear, however, sufficiently promising to warrant its continuation at the time.

An extensive investigation of plant pectins is at present being carried out in this laboratory by Dr. Schryver. It is hoped that on the basis of his results it may be possible to elaborate a suitable chemical method for standardizing fungal extracts as regards their capacity to dissolve the cell-wall.

is, however, quite unsafe, as there is no guarantee that the discs remain unaltered when so preserved. In point of fact, observations have shown that alterations do take place. Thus, if a number of discs from the same region of the same potato are preserved in different antiseptics, they are found after a week's interval to show widely different degrees of sensitiveness to the action of the fungal extract. Variations amounting to as much as 400 per cent. have in this way been produced. A more striking example of the same phenomenon is given later (p. 344). The above serves to illustrate the dangers attendant upon this method of standardizing extracts.

## E. ACTION OF EXTRACT ON TISSUES.

(a) *General Account.*

The action of the extract is of a twofold nature:

1. Solution of certain constituents of the cell-wall, resulting in loss of coherence of the tissue.

2. Death of the cells themselves.

These two aspects of the phenomenon will in the meantime be referred to respectively as the 'macerating' and 'lethal' actions of the extract.

Fleshy tissues were prepared in the way described in the preceding section. The following tissues were tested and found to be readily acted on: Tubers of *Potato*, roots of *Turnip*, *Beet*, *Radish*; fruit tissue of *Apple*, *Cucumber*; pith of stem of *Senecio articulata*. With extract of normal strength, potato discs of  $\frac{1}{2}$  mm. thickness are very usually disintegrated in twenty to thirty minutes, though, as has been stated, there may be considerable individual variation. Discs of white or yellow Turnip require as a rule a similar time, while the harder tissue of Swedes is more slowly acted upon. The above list is no doubt capable of wide extension, and it is indeed highly probable that the fleshy parenchymatous tissue of fruits, tubers, &c., is very generally susceptible to the action of this fungus extract.

Tissues of leaves, petals, &c., were treated in a variety of ways. Discs of these were submerged in the active extract, so that action proceeded from the margin (the cuticle presenting an impenetrable obstacle to the diffusion of the extract, as will be shown in a subsequent paper), or the extract was injected into the tissue by means of the air-pump. In a series of experiments the extract was injected into the tissue by means of a hypodermic syringe. The advantage of this method is that the tissues are maintained in a more normal condition during the course of the action than when they are submerged in liquid.

When the discs are merely submerged in the extract the action may be comparatively slow, being to a large extent limited by the rate with which the leaf disc becomes injected. This injection process, which also takes place when the discs are immersed in water, is somewhat obscure in principle;<sup>1</sup> in particular the fate of the air of the intercellular spaces is not quite clear. Injection proceeds chiefly along the line of the vascular bundles, especially from the proximal end. The course of the action is also influenced by the mechanical properties of the leaf discs. Thus, in the case of tulip petal discs, the disintegration of the more sensitive central tissue

<sup>1</sup> This phenomenon is probably related to that of the ascent of water in shoots which are kept in a saturated atmosphere. The injection would thus be due to an active pumping action on the part of the cells of the leaf; cf. Dixon, Proc. Roy. Ir. Acad., vol. iv, 1896-8, p. 627.

along the cut surface results in the rolling apart of the upper and lower halves, so that the extract rapidly gains access to all parts of the disc; in rose petal discs the same effect is produced by the rolling back of the lower cuticle.

When the active extract is injected into the tissue, the action may be very rapid. The most marked effects are seen in the case of floral structures, in which injection of the extract produces rotting and death within half an hour.<sup>1</sup> This has been found to be true of upwards of thirty species investigated, whence it would appear that the extract is destructive to floral structures in general. In the case of foliage leaves, the rate of action is as a rule much slower, but is nevertheless strongly marked in many cases—leaves of succulents, of *Vicia Faba*, *Viola*, *Petunia*, *Lactuca*, *Begonia*, &c. In the case of leaves of a hard woody nature it has not been shown in any case that the extract has any action. Thus leaves of *Aucuba*, pitchers of *Nepenthes*, have afforded negative results.

When tissues of lower plants were investigated in this respect, unexpected results were obtained. Nordhausen (l.c.) records certain experiments in which moss leaves showed themselves very sensitive to the action of *Botrytis*, and it was in view of this statement, as also on account of their softness, that tissues of Bryophytes were expected to succumb rapidly to the action of the extract. Quite the opposite result was obtained, and in fact it is not too much to say that these forms show complete resistance to the action of the fungal extract. The following have been investigated:

Thallus of *Pellia*, *Fegatella*; leaves of *Plagiochila*, *Funaria*, and *Mnium*.

In no case whatever was any definite alteration demonstrated, even after several days' action of the extract, the latter being renewed from time to time.<sup>2</sup>

In view of this startling discrepancy, experiments were set up to see if any confirmation could be obtained of Nordhausen's statements as to the action of the fungus itself. For this purpose small clumps of the above-mentioned plants were sprayed with a turnip extract suspension of *Botrytis* spores, which were then allowed to germinate. In the course of a few days the clumps were quite infested with the fungus, becoming in fact totally covered and hidden by the mass of mycelium. Even after this drastic treatment, the plants, both mosses and hepatics, were found to be only very slightly affected, appearing slightly limp and unhealthy, but showing no

<sup>1</sup> In the case of the soft petals of *Gloxinia*, *Achimenes*, *Tradescantia*, *Saintpaulia*, &c., the effect of the extract is apparent within five minutes of the time of injection. Further action of the extract leads to almost complete solution of these petals.

<sup>2</sup> In the case of the moss leaves examined, groups of cells here and there were seen to be discoloured, but in no case was anything like a general action of the extract shown. Similar and, as far as could be judged, equal discolorations were seen in the control moss leaves which had been kept in water.

rotting nor any invasion by the fungus. The slight effects observed were set down to bacterial contamination, the cultures having become by this time very foul. In another experiment, pieces of *Pellia* thallus, leaves of *Mnium* and *Plagiochila* as well as portions of various higher plants (leaves of *Geranium*, *Dahlia*, petals of ditto) were placed on a plate of germinating spores, set up as previously described, control pieces being placed on a similar plate from which the spores were absent. In the course of twenty-four hours the *Geranium* and *Dahlia* tissues were largely invaded and decomposed; after forty-eight hours they were entirely rotten, while the controls were still unchanged. On the other hand, up to the end of the experiment, which lasted for four days, the moss and hepatic tissues appeared quite unaffected, although they had been completely overrun by the fungus.

The conclusion from these experiments, viz. that mosses and hepatics show a considerable degree of resistance to the attack of *Botrytis*, seems more in accordance with experience than the results of Nordhausen. The habitat of these plants is one which would be calculated to encourage the growth of the fungus. If they are as highly susceptible to attack by the fungus as Nordhausen states, it is surprising that no natural occurrence of the latter on these plants has been described. Apart from the experiments of Nordhausen, there appears to be no case of infection of mosses by *Botrytis* recorded.<sup>1</sup>

Among Algae, the action of *Botrytis* extract was tested on filaments of *Spirogyra*. From the two tests made in this connexion it appeared doubtful whether any action occurred. If any, it was certainly very slight. Here by way of contrast it may be mentioned that experiments with the filamentous staminal hairs of *Tradescantia virginica* gave a strong positive result, the filaments rapidly breaking up into the individual cells, from which the coloured contents soon became discharged.

Viewed generally, the macerating action of the extract shows itself in the tissue losing completely its coherence, subsequently breaking down to form a mush, and in extreme cases passing almost completely into solution. With regard to the lethal action, the criteria must to a large extent be chosen to suit the particular circumstances. In the case of such tissues as turnip or cucumber, the lethal action of the extract can be demonstrated by failure of the cells after a time to show plasmolysis in hypertonic solutions.

<sup>1</sup> The contradiction is in certain respects not absolute. Nordhausen took leaves of *Mnium*, laid them on the surface of a nutrient jelly, and sprayed *Botrytis* spores over the upper surface. The spores grew down into the cells of the leaf, being attracted chemotropically by the nutrient passing up from the underlying layer of jelly. The moss cells were killed, their contents discoloured, and the cell cavities finally became filled with a mass of hyphae. On the last point it is difficult to see how any mistake could have arisen, and it may be that the absence of a special chemotropic factor in the experiments of the present paper may have been responsible for failure to produce infection.

In coloured cells, death is as a rule shown by escape of the coloured substance. This substance may remain as such and impart its colour to the fungal extract (e. g. Beet), but more generally it disappears from view altogether (e. g. Rose, *Viola*, and many other petals), though its presence may be demonstrated by the addition of a suitable reagent (e. g. an acid in the case of the red pigment of Rose petals). In other cases the incidence of death is shown by a new development of colour, due to autolysis, i. e. to actions taking place which were held in abeyance during life. Such is the well-known black coloration produced after death in the leaf of the Broad Bean.

These post-mortem phenomena as witnessed in the various tissues experimented upon do not call for detailed description here. It is important, however, to state that the post-mortem changes brought about by the fungal extract, with one exception which is only apparent,<sup>1</sup> were identical with those induced by the action of the fungus itself. Again in all cases, where a distinct parasitism of the fungus on a particular host could be established, it was found that the tissues of the latter were acted upon in a similar way by the fungal extract. These considerations—viz. similarity of 'range', and similarity of effects produced—justify the conclusion that the standard extract of the present investigation is a true representation in essentials of the active principle of the fungus.<sup>2</sup> It is proposed, therefore, on the basis of these results, to put forward the following as a working hypothesis—that all the macerating and lethal effects of the fungus can be explained on the basis of the properties of the standard fungal extract.

<sup>1</sup> In the case of the leaf of *Vicia Faba*, it was found that the discs, when injected and submerged in a quantity of fungal extract, did not show the characteristic blackening produced by the fungus itself, but remained for a considerable time quite green, fading later into yellow. There was no doubt as to the discs being killed, for within a few hours from the commencement of the experiment they became quite limp and rotten, and the cells were no longer capable of plasmolysis. Moreover, no blackening effect appeared if these discs were subsequently placed in chloroform vapour. It was by reason of this discrepancy that the hypodermic syringe method was first resorted to, when it was found that leaves injected with extract according to this method gave the normal blackening. The explanation of these appearances is fairly simple. The black pigment is formed as the result of an oxidase reaction, in which the source of oxygen is the free oxygen of the atmosphere. The latter is available in the case where the leaf is injected by means of the hypodermic syringe; not so when it is injected and immersed in some depth of liquid. In the latter case, at the time and place of killing, one of the factors essential for the development of the black colour is to a large extent wanting; the two remaining factors concerned (enzyme and oxidizable substance) diffuse out into the liquid, where they gradually meet the atmospheric oxygen, so that a development of the black pigment takes place slowly in the liquid. This last had, in fact, been noticed long before the apparent discrepancy was understood.

<sup>2</sup> In Smith's experiments, treatment of lettuce leaf with oxalic acid produced a bleaching effect, in contrast with the browning effect produced by the fungus and fungal extract. These results have been confirmed, and in the opinion of the present writer this disparity in post-mortem effects constitutes a strong objection to the view that oxalic acid is the toxic principle of the fungal extract and of the fungus.



*(b) Detailed Account of certain Cases.*

As has been stated, the action of the fungal extract on a tissue is of two kinds, a macerating and a lethal. The following account refers to an examination of the course of the action which was carried out with the object of establishing the time relationships of the two manifestations.<sup>1</sup> The criterion of maceration was as usual diminution of coherence. The criterion of lethal activity was the failure of hypertonic solutions to cause plasmolysis.

It will be noted that of the two phenomena to be studied, the one entails microscopic, the other (in the main) macroscopic observation. In order to make correlation possible it is obvious that homogeneity of tissue is all-important. We are thus limited to fleshy parenchymatous tissue. Even here the ideal tissue is not obtainable. Thus in turnip tissue homogeneity is disturbed by the presence of small vascular bundles, the small cells in the neighbourhood of which are more slowly acted upon than the larger cells of the ground-mass. In other cases—e. g. in the stem (pith) of *Senecio articulata*—there is a gradation from the large, more sensitive cells in the centre to smaller, more slowly reacting cells towards the periphery. The difficulty, however, is not so great as might appear. The coherence of a disc of any particular tissue is no greater than that of its weakest part, and we have accordingly to compare the progress of the macerating action, as shown by the loss of coherence, with the plasmolytic features of the more sensitive portions. Thus in turnip tissue we correlate the condition with respect to coherence with the condition as regards plasmolysis of the large cells of the ground-mass; and similarly for the case of the large central cells of the pith of *Senecio articulata*.

The tissues which have been found useful in this connexion are as follows:<sup>2</sup>

*Turnip (white)*: nearly full grown; must not be old and vacuolated; discs cut from an axial cylinder in the neighbourhood of the centre.

*Swede*: possesses the great advantage that uniform discs of  $\frac{1}{2}$  mm. thickness can be cut.

*Cucumber*: discs from axial cylinder in the region of the basal contracted portion.

<sup>1</sup> Van Hall (l. c., p. 135), from microscopic examination of the tissue lying between the sound and the decomposed tissue, states 'that it is sometimes possible to observe one or two layers of tissue, the cells of which still hang together, although, as can be seen from their contracted protoplasts, they are already dead'. From this he concluded that the cells are first killed and then isolated from each other.

<sup>2</sup> The potato, which has proved of great value in this investigation, is useless for the present purpose on account of the large amount of solid cell contents present, which makes a plasmolytic study impracticable.

*Senecio (Kleinia) articulata*: Transverse sections of stem, the sides being cut away so that the sections take the form of narrow strips including the central tissue of the stem.

*Cotyledon rosacea*: Transverse sections of leaf, prepared in the same way as the immediately preceding.

In all cases the tissue discs or strips were prepared according to the method for quantitative experiment; previous to use they were injected with water.

The following account of an experiment with *transverse axial strips of pith of Senecio articulata* will serve to illustrate the sequence of phenomena observed:

Strips placed in standard extract at 4.5 p.m. 4.20, strips coherent; ditto at 4.25 and 4.30. 4.35, coherence gone.

A strip at this stage is placed for three minutes in a 5 per cent. solution of  $\text{KNO}_3$  in which a little eosin is dissolved; then washed in 5 per cent. solution of  $\text{KNO}_3$ . On microscopic examination, this strip shows as many fully plasmolysed cells in the central region as a strip which has not been exposed to the action of the extract. The cells of the strip exposed to the extract only differ in appearance from those of the latter inasmuch as the cell-walls do not appear so well defined and are more transparent. There is no marked swelling of the walls.

If a strip which has been treated with extract be pulled apart at this stage, it shows a characteristic appearance along the line of separation. Separation follows the line of the cell-walls, the cells on either side being left intact. When, on the other hand, a strip of tissue which has not been treated with extract is pulled apart at the centre, the line of separation passes as often as not across the cells so that the tissue at the margin possesses a ragged appearance. This phenomenon is taken as indicative of the action of the extract upon the middle lamella, and it shows that the latter is in an advanced state of solution at a time when the cells are still alive and when the remaining portions of the cell-wall still possess their mechanical properties.

4.50—a strip on plasmolysis still shows a large proportion of live cells in the central region, quite close up to the line where the strip separates on being pulled. The tissue is now very difficult to handle without producing damage. Thus when a cover-glass is laid on a portion of the central tissue, the latter becomes a disorganized mush. The collapsed cell-walls of this mush show a flaky or layered appearance, indicating solution of certain cell-wall constituents, a process which results in the cell-wall losing its mechanical properties.

5.5 and 5.20—a fair proportion of central cells still plasmolysable.

5.50—central region now shows only occasional living cells, the protoplasts of which are reduced by plasmolysis to very small globules; the

tissue is now to a large extent a mush even without the disturbance incidental to the addition of a cover-glass.

Thus in the present case, while the tissue coherence becomes reduced to a negligible quantity in thirty minutes, the cells themselves in the most sensitive region are all alive at this stage, and a certain proportion are still alive after seventy-five minutes.

The following represents a summary of similar experiments in this connexion :

*Turnip (white) discs*: coherence gone in 15'; considerable proportion of plasmolysable cells after 20'; this proportion much reduced after 30'.

*Cucumber discs* (weak extract employed): coherence gone in 25'; large proportion of plasmolysable cells after 45'; this proportion much reduced after 60'; only an occasional plasmolysable cell after 90'.

*Turnip (white) discs* (weakened extract): coherence gone in 90'; proportion of plasmolysable cells undiminished after 120'; proportion distinctly decreased after 135', but a fair percentage of plasmolysable cells still remains after 150'.

*Turnip (Swede) discs* of  $\frac{1}{8}$  mm. thickness (weakened extract): coherence gone in 40'; proportion of plasmolysable cells undiminished at this stage, also at 50' and 55'; slight reduction after 60', but still considerable proportion of live cells after 70'; number of plasmolysable cells small after 90'.

As comparable with the preceding may be cited certain experiments with the staminal hairs of *Tradescantia virginica*. These are rapidly disintegrated by the fungal extract, so that after a time it is easy by gentle shaking to cause them to break up into their individual cells; if treated at this stage with 5 per cent.  $\text{KNO}_3$  the cells are found to be almost universally plasmolysable.

On the other hand, cases were found where this time separation of macerating and lethal effects could not be effected. This was the case with sections from the fleshy leaf of *Cotyledon arborea*, and also to a less extent with sections from old white Turnip. Here it was found that at the stage where coherence was lost considerable disintegration of the cell-walls could be demonstrated and a large proportion of the cells were no longer plasmolysable.

Summing up, the action of the extract on living tissue is as follows :

The first noticeable change is the solution of the middle lamella, so that the tissue loses its coherence. At the stage which is termed 'coherence gone', this action has progressed so far that the middle lamella has lost its mechanical properties as a solid layer 'cementing' the cells of the tissue together. As a consequence the tissue readily falls apart along the line of the middle lamella. At this stage, however, the remaining layers of the cell-wall possess in some degree their original mechanical properties, and the cells themselves are quite alive. Very soon the remainder of the cell-wall is disintegrated, breaking down into what appear to be flakes or

lamellae, and the whole structure becomes very fragile. This feature becomes more and more pronounced, and in course of time the tissue falls into a 'mush'. In no case has complete solution of the cell-wall been seen. Death of the cells takes place some time after fragmentation of the cell-walls is definitely established; the latter process, in the majority of cases examined, is not in evidence at a time when the tissue has lost all coherence as a result of the solution of the middle lamella.

#### F. EXAMINATION OF CERTAIN PHYSICAL RELATIONSHIPS OF EXTRACT.

This investigation was undertaken in the first instance with the object of trying to effect a separation between the 'macerating' and 'lethal' principles of the extract. As the research proceeded it was, however, found expedient to develop it on broader lines. This examination in certain parts is not yet completed.

##### *Relation to Heating.*

*The activity of the extract, both as regards macerating and lethal effects, is totally destroyed by a sufficient degree of heating.*

Below 55° C. deactivation is comparatively slow; above this temperature it becomes very rapid, and at 65° it is as near as may be instantaneous. To study the effect of heating between the limits of 50° and 65°, the following method of heating was adopted by way of standard:

A small tube containing 2 c.c. of extract was dipped into a beaker of water which was kept at a temperature 5° higher than the temperature desired. A thermometer served the double purpose of stirring the contents of the tube and recording the rise of temperature. The tube containing the extract was taken out immediately the required temperature was reached. This heating process, for temperatures between 50° and 65°, occupies approximately half a minute.

The following table shows the rapid nature of heat deactivation when a temperature of about 55° is reached. The heating process was carried out in each case as described above.

'Macerating activity' = reciprocal of time required to cause loss of coherence, that of unheated extract being taken as unity.

##### *Extracts tested on Turnip Discs.*

|           | Treatment.    | Macerating activity. |
|-----------|---------------|----------------------|
| Heated to | 50° . . . . . | 1                    |
| "         | 53° . . . . . | 1 —                  |
| "         | 55° . . . . . | $\frac{2}{3}$        |
| "         | 58° . . . . . | $\frac{1}{2}$        |
| "         | 60° . . . . . | $\frac{1}{10}$       |
| "         | 63° . . . . . | $\frac{1}{100}$      |
| "         | 65° . . . . . | 0                    |

At and below 50° deactivation is relatively slow, as is shown by the following figures:

| Treatment. |                 | Macerating activity. |      |
|------------|-----------------|----------------------|------|
| Heated at  | 50° for 15 min. | .                    | 1/18 |
| "          | 50° " 45 min.   | .                    | 1/12 |
| "          | 45° " 1 hour    | .                    | 1/8  |
| "          | 40° " 1 hour    | .                    | 1/6  |
| "          | 35° " 1 hour    | .                    | 1    |

In all of the above cases where deactivation of the macerating principle was partial, it was seen that no separation of macerating and lethal principles had been effected. The lethal effect was tested in the case of the stronger solutions by injections into bean leaves; the weaker (much deactivated) extracts were tested as to their killing action on the more sensitive petals of *Gloxinia*. In all such cases a lethal action could be demonstrated. With extracts which had been completely deactivated in respect of their macerating action by heating to 65°, the lethal action was also completely stopped. This point is illustrated by the following experiment:

Two discs of *Crocus* petal were injected under the air-pump, the one in unheated extract, the other in extract which had been heated to 65°. Both extracts had been previously cooled to nearly 0° C., and throughout the experiment were kept in an ice chest. The disc injected with unheated extract was completely disorganized in an hour: that in the other was alive and apparently unaltered after five days, at which time, as bacteria were now beginning to appear in numbers, the experiment was discontinued.

Throughout this work, extract which has been heated in the manner described to 65° has been used as the standard control.

It is noticeable that heat deactivation causes a certain amount of coagulation in the extract, which is seen to become more opalescent. This, however, has nothing to do with the phenomenon of deactivation, as the extract when purified in certain ways shows the same sensitiveness to heating, but without exhibiting any coagulation.

#### *Relation to Mechanical Shaking.*

The extract may be deactivated by mechanical agitation—e.g. by bubbling air through it or by shaking in a closed vessel. As it is proposed to publish the results of this investigation separately, no detailed description will be attempted here. The following figures illustrate the magnitude of the effect:

(Agitation produced by a stream of bubbles.)

Activity of extract maintained (but not shaken) at 35° for 1 hr. = 1.

" " " shaken at 35° for  $\frac{1}{2}$  hr. =  $\frac{2}{5}$ .

" " " shaken at 35° for  $\frac{3}{4}$  hr. =  $\frac{1}{5}$ .

As in the case of heat deactivation, it was found that no separation of macerating and lethal actions could be effected by this means.

*Relation to Diffusion and Dialysis.*

The experiments on this subject are not yet completed, and it is therefore proposed to give certain results only in *résumé*.

Comparative diffusions were carried out by means of a series of graded gelatine (5, 10, 15, 20 per cent.) membranes prepared after the method of Bechhold. From these experiments it appeared that the macerating principle possessed a coefficient of diffusion comparable with that of *dextrin* and greater than that of *diastase*. It is thus a colloid of intermediate type. Again, all the fungal extracts which have been purified by diffusion through gelatine membranes showed a normal lethal activity, and though it has not been possible as yet to perfect the experiments in this connexion, there is a strong presumption that if the diffusate possesses any macerating activity, it possesses likewise lethal activity—in other words, if there are two principles concerned, the diffusive capacity of the lethal principle is not less than that of the macerating one.

A complete dialysis of the macerating principle was effected by the use of a certain type of collodion thimble. In this case it was found that the macerating principle could be completely held back. On testing the dialysate, after removal by means of the air-pump of the volatile antiseptic, no trace of a killing action was shown. Subsequent control experiments with the same membranes showed that they were quite permeable to crystalloids such as cane sugar, ammonium oxalate, &c.

By this dialysis method a very convincing proof was obtained that soluble oxalates do not play any part whatever in the lethal activities of the extract. The dialysate from the collodion thimbles gave a precipitate with potassium oxalate solution, thus showing the presence of a substance (presumably a calcium salt) which precipitated oxalates. Control experiments showed that this substance was not derived from the membrane itself. It was accordingly present in solution in the continuous phase of the colloidal extract, so that the simultaneous presence of a soluble oxalate was quite excluded.<sup>1</sup>

<sup>1</sup> This dialysis experiment was in one case carried out with the following precautions: The collodion membranes were never allowed at any time to come into contact with tap-water. The germinated spores were subjected to prolonged washing in distilled water and dried *in vacuo* over sulphuric acid. The dried material was ground without sand, a control experiment having shown that no calcium was derivable from the mortar. With extract from this material it was also found that calcium could be demonstrated in the dialysate. Since these precautions for excluding contamination with traces of calcium were not usually adopted, it is safe to assume that the presence of a soluble calcium salt can be predicated of all the extracts that have at different times been employed. Whether the calcium salt is derived from the contents of the fungus cells or is strongly adsorbed on the walls from the liquid in which germination took place, it is impossible to say.

It appears, therefore, that by none of the methods above described was it possible in any way to obtain any separation of the macerating and lethal actions of the extract.

#### G. RELATION OF ACTIVITY OF EXTRACT TO CERTAIN CHEMICAL SUBSTANCES.

##### *Acidity of Extract.*

The standard extract shows a weak acid reaction. With any of the usual indicators, the neutral point is not sharply defined, so that the acidity can only be measured approximately. With phenolphthalein,  $\frac{n}{160}$  was established as upper limit of acidity; with neutral red the acidity was given as  $\frac{n}{180}$  to  $\frac{n}{200}$ . During the process of neutralizing the latter indicator changes continuously from red to yellow. This would indicate that the acids present are of a very weak or of a polybasic nature.

##### *Effect of varying the Acidity on the Activity of the Extract.*

(i) *Diminution of Acidity.* As the acidity of the extract is diminished, the activity remains unaltered up to a certain point, when it falls sharply to zero. In alkaline solution the extract has no activity. The following table will serve to illustrate the sharp effect produced on neutralization of the extract:

Indicator in each case = 1 drop of  $\frac{1}{10}$  per cent. Solution of Neutral Red.  
20 drops of Alkali solution = 1 c.c.

| No. | Extract.  | Indicator.                   | Acidity.              | Time to decompose turnip disc. |
|-----|---|------------------------------|-----------------------|--------------------------------|
| 1.  | 5 c.c. Ext. + 6 drops water                       | red                          | $\frac{n}{200}$       | 18 min.                        |
| 2.  | 5 c.c. Ext. + 4 " " + 2 drops $\frac{N}{10}$ NaOH | grading                      | $\frac{3n}{1000}$     | 18 min.                        |
| 3.  | 5 c.c. Ext. + 3 " " + 3 " "                       | continuously                 | $\frac{2n}{1000}$     | 20 min.                        |
| 4.  | 5 c.c. Ext. + 2 " " + 4 " "                       | into                         | $\frac{n}{1000}$      | 35 min.                        |
| 5.  | 5 c.c. Ext. + 1 " " + 5 " "                       | { clear, permanent<br>yellow | o                     | 100 min.                       |
| 6.  | 5 c.c. Ext. + 0 " " + 6 " "                       |                              | $\frac{n}{1000}$ alk. | > 20 hrs.                      |

In the case of (6) the disc remained coherent and quite alive (i.e. turgid) after 20 hours. In the remainder, death of the cells followed in the usual way. This shows that the lethal principle of the extract reacts as

sharply and at the same point to the addition of alkali as does the macerating principle.<sup>1</sup>

When the concentration of alkali is further increased, a macerating action again sets in. Control experiments show that this is due to the alkali itself. With potato tissue a macerating action sets in when the concentration of alkali approaches the value  $\frac{n}{50}$ : with tissue of white

Turnip a concentration of  $\frac{n}{100}$  alkali can be seen to produce a slow macerating effect. It is noteworthy that maceration by dilute alkali is also accompanied by death of the cells.

The active principle is inhibited but not destroyed by the alkali. On adding an appropriate amount of acid, the activity of the extract is restored, that is, apart from the retarding action of the salt thereby produced.

(ii) *Increase of Acidity.* In this connexion the following acids have been tested—*citric, malic, tartaric, sulphuric, and hydrochloric*. In the case of the two mineral acids and of the dilute concentrations of the organic acids, the required concentration of acid in the extract was obtained by adding a measured quantity (in drops) from a strong solution of appropriate strength. The slight degree of dilution of the principle of the extract which is thereby produced is known to be of no importance. The higher concentrations of the organic acids were obtained by evaporating solutions of the strength desired to dryness on the water bath, and making up to the original volume with standard extract.

In all cases the action of the acid on the activity of the extract is one of retardation. The retarding effect in relation to normality is approximately equal for all the acids up to a certain point ( $\frac{n}{32}$  to  $\frac{n}{64}$ ); above this point the retarding action for the mineral acids increases very much more rapidly than that of the organic acids. In the case of the mineral acids, the macerating action of the acid appears at a fairly low concentration, so that above this point the action of the acid extract is not distinguishable from that of the acid itself. In the case of the organic acids macerating effects due to the acid appear at a much greater concentration, so that the retarding action of the acid upon the extract can be studied over a comparatively wider range.

<sup>1</sup> As the colour reaction is not sharp, it may well be that (6) represents the neutral point more closely than does (5). Furthermore, there is an escape of acid juice from the turnip disc, so that the acidity of the extract varies with time, especially in the neighbourhood of the neutral point. Thus the activity assigned to extract (5) in the preceding table is probably too high, or, more exactly, the activity of that extract increases with the gain in acidity due to the escape of acid sap from the turnip disc. It is therefore impossible to say whether the activity of the extract disappears when the neutral point is reached or whether deactivation is completed when the solution reacts alkaline.



The following tables illustrate the above statements :

(a) In dilute concentrations (up to  $\frac{n}{64}$ ), there is considerable agreement among the acids employed: the retarding effect of each increases gradually with the concentration. The figures represent the time in minutes required to produce loss of coherence in potato discs.

| Acid.        | $\frac{n}{\infty}$ | $\frac{n}{1024}$ | $\frac{n}{512}$ | $\frac{n}{256}$ | $\frac{n}{128}$ | $\frac{n}{64}$ |
|--------------|--------------------|------------------|-----------------|-----------------|-----------------|----------------|
| Citric       | 20-25              | 20-25            | 25              | 30-35           | 35              | 35-40          |
| Malic        | 20-25              | 20-25            | 25              | 25-30           | 35              | 35-40          |
| Tartaric     | 20-25              | 20-25            | 25              | 30-35           | 35              | 35-40          |
| Sulphuric    | 20-25              | 20-25            | 25              | 30-35           | 35-40           | 40             |
| Hydrochloric | 20-25              | 20-25            | 25-30           | 30-35           | 35-40           | 40             |

(b) In the case of sulphuric and hydrochloric acids, the retarding action increases very rapidly above a concentration of about  $\frac{n}{32}$ .

| Acid.                          | $\frac{n}{\infty}$ | $\frac{n}{128}$ | $\frac{2n}{128}$ | $\frac{3n}{128}$ | $\frac{n}{32}$ | $\frac{n}{17}$ | $\frac{n}{12}$ | $\frac{n}{9}$ | $\frac{n}{7}$ | $\frac{n}{6}$ | $\frac{7n}{38}$ |
|--------------------------------|--------------------|-----------------|------------------|------------------|----------------|----------------|----------------|---------------|---------------|---------------|-----------------|
| H <sub>2</sub> SO <sub>4</sub> | 20-22              | 30-35           | 35               | 35               | 90-105         | ⊕              | ⊕              | +             | +             | +             | +               |

⊕ These discs and those of the acid controls do not lose coherence within 24 hours.

⊕ These discs lose coherence, the more rapidly the higher the concentration of acid; equal effects are shown by the acid controls.

Hydrochloric acid shows very similar effects: thus a concentration of  $\frac{n}{15}$  stops the macerating action of the extract.

(c) The retarding action of the organic acids above the concentration  $\frac{n}{32}$  is much less than that of the mineral acids :

| Acid.    | $\frac{n}{\infty}$ | $\frac{n}{160}$ | $\frac{n}{40}$ | $\frac{n}{10}$ | $\frac{n}{5}$ | $\frac{2n}{5}$ | $\frac{3n}{5}$ | $\frac{4n}{5}$ | $n$           |
|----------|--------------------|-----------------|----------------|----------------|---------------|----------------|----------------|----------------|---------------|
| Citric   | 20                 | 30              | 40-45          | 40-45          | 45            | 75-90          | 120-135        | 210            | ⊕             |
| Malic    | 20                 | 30              | 40-45          | 40-45          | 45            | 75             | 90-105         | 135-150        | > 4 < 18 hrs. |
| Tartaric | 20                 | 35              | 40-45          | 40-45          | 45            | 135-150        | > 18 < 30 hrs. | ⊕              | ⊕             |

⊕ Discs do not lose coherence in 3 days.

In all these acids the retarding action is approximately equal and is not considerable, up to  $n/5$ ; above this concentration it increases more rapidly. The specific retarding action is least in malic and greatest in tartaric acid.

#### *Effect of Salts and other Substances on the Activity of the Extract.*

*Salts.* Here, as in the case of acids, the action is one of retardation. The variation of the retarding effect with concentration was followed in detail in the case of potassium nitrate. The magnitude of the retarding effect will appear from the following figures, potato discs being employed.

|     | $\frac{m}{\infty}$ | $\frac{m}{572}$ | $\frac{m}{256}$ | $\frac{m}{128}$ | $\frac{m}{64}$ | $\frac{m}{32}$ | $\frac{m}{16}$ | $\frac{m}{8}$ | $\frac{m}{4}$ | $\frac{m}{2}$ |
|-----|--------------------|-----------------|-----------------|-----------------|----------------|----------------|----------------|---------------|---------------|---------------|
| (1) | 15                 | 15              | 15              | 15+             | 25             | 40             | 75             | 180           | 3½-4 hrs.     | 10-20 hrs.    |
| (2) | 15                 | 15              | 15              | 15+             | 30             | 45             | 90             | —             | —             | —             |

The amount of agreement obtained in experiments of this sort may be judged from comparison of the results of the two experiments tabulated above. A similar investigation with respect to KCl gave retarding effects somewhat less than those of  $\text{KNO}_3$ .

A detailed investigation of a variety of salts was not attempted. A certain number were, however, tested in this respect in order to see if the effects were in any way general; and in particular a salt of calcium was included in the list with a view to determining if it exerts any special action upon the extract, seeing that calcium is known to be a constituent of the middle lamella. The figures are given in the table below. In the case of each salt solution employed, it was previously ascertained that it showed a neutral reaction (this precaution must be particularly exercised with solutions of calcium chloride, which are liable to contain free alkali).

*Time for Standard Extract = 17-20'.*

| Salt.                    | $\frac{m}{64}$ | $\frac{m}{16}$ | $\frac{m}{4}$ and $\frac{m}{2}$ |
|--------------------------|----------------|----------------|---------------------------------|
| NaCl                     | 60             | 165            | x                               |
| KCl                      | 20+            | 70             | x                               |
| $\text{NH}_4\text{Cl}$   | 20+            | 135            | x                               |
| Na-Ac                    | 70             | 90             | x                               |
| $\text{K}_2\text{Ox}$    | 50             | x              | x                               |
| $\text{Na}_2\text{SO}_4$ | 40             | 90             | x                               |
| $\text{K}_2\text{SO}_4$  | 70             | 115            | x                               |
| $\text{MgSO}_4$          | x              | x              | x                               |
| $\text{CaCl}_2$          | 50             | x              | x                               |

x Coherent after 3 hours; devoid of coherence after 20 hours.

A subsidiary experiment with the last three salts showed that in no case were the retarding effects due to alteration of the tissue by the salts employed.

As expected, different salts show marked variation in their retarding actions. It is not proposed to draw any general conclusions from the above figures, as it has not been thought fit for the present to pursue this subject intensively. It appears, however, that no special action is to be ascribed to the calcium salt in this connexion. Also, the strong retarding effect of the magnesium sulphate is noteworthy.

Of other *crystalloidal neutral substances*, only two have been investigated—saccharose and glucose. No definite retarding effect has been demonstrated in the case of these.

*Time for Original Extract = 30'.*

|                | $\frac{m}{64}$ | $\frac{m}{16}$ | $\frac{m}{4}$ | $m$   |
|----------------|----------------|----------------|---------------|-------|
| $\text{KNO}_3$ | 55             | 150            | 10 hrs.       |       |
| Saccharose     | 30             | 30             | 30-35         | 30-35 |
| Glucose        | 30             | 30             | 30-35         | 30-35 |

While it would appear that the retarding action of a chemical substance is related to its capacity of ionization, it is still quite possible that some non-ionizable substances may exert a specific retarding or even an *anti*-action upon the principle of the fungal extract. In this connexion one may suggest such substances as tannins, alkaloids, latex constituents, &c. In any investigation of immunity from the biochemical side, experiment along these lines might prove to be of value. As, however, the specialization in relation to host is very slight in the case of the fungus under consideration, such a detailed investigation has not been attempted in the present case.

Traces of alcohol, chloroform, acetone, and formaldehyde do not, as far as can be seen, have any appreciable effect upon the macerating action of the extract.

*Colloidal substances* might be expected to influence the activity of the extract by disturbing the adsorptive equilibrium within the liquid. In this connexion only one colloid has been tested—viz. starch. The investigation of this point arose incidentally in the examination of the effects of dilution on the activity of the extract. An account of these experiments may conveniently be given here.

Three diluents were used: (1) water, (2)  $\frac{1}{2}$  per cent. starch solution, (3) extract deactivated by heating to 65°. The figures in the table represent the times required to produce loss of coherence in potato discs.

| Concentration. | Diluent. |                                  |                      |
|----------------|----------|----------------------------------|----------------------|
|                | Water.   | $\frac{1}{2}$ % Starch Solution. | Deactivated Extract. |
| 1              | 20       | 20                               | 20                   |
| $\frac{1}{2}$  | 25       | 25                               | 25                   |
| $\frac{1}{4}$  | 40       | 40                               | 35                   |
| $\frac{1}{8}$  | 65       | 65                               | 45                   |

From this it appears that the starch has no measurable effect in the concentration employed. The greater activity of the extract diluted according to the third method is somewhat interesting. It would appear to be connected with the maintenance of the original acidity, the acidities of the extracts diluted with water or with starch solution being reduced very nearly to zero. (See p. 338, on effect of reduction of acidity on activity.)

From the fourth column of the above table we see that the activity of the extract is proportional to the square root of the concentration of active principle. In the neighbourhood of the full strength of the extract (i. e. with 'standard' extract) there is considerable divergence from this law. Here the decrease in activity on dilution is less than required by the law, or conversely the gain in activity with increase of concentration becomes negligible after a certain concentration is reached. This is in agreement with what was mentioned in dealing with the technique of extraction, where

it was stated that extracts of greater concentration than the standard did not appear to possess any increased activity. This asymptotic effect suggests that some other limiting factor comes into play when the higher concentrations are reached. Such a factor may be the rate of diffusion of the active principle into the tissue of the discs experimented with. The above considerations also show how it is that in certain cases a better comparison of the strengths of two extracts may be obtained by using the diluted in place of the extracts of full strength.

*Influence of Plant Juices on Activity of Extract.*

That plant juices retard the action of the fungal extract was known at an early stage of this investigation, and in fact this knowledge formed the starting-point of the experiments which have just been described. A series of experiments was now carried out to see if there is much difference among various plant juices in respect of the effect they produce upon the fungal extract; and more particularly, to see if any parallelism could be traced between the specific retarding effect of the plant extract and the relative immunity of the plant itself to the action of the fungus or of the fungal extract. It was known that a high degree of resistance to the action of the extract was shown by tissues of mosses and hepatics; and it was therefore of considerable interest to determine if this want of activity of the fungal extract was due to its deactivation by some specific anti-body capable of extraction from the resistant tissue.

The plant juices were prepared without dilution by squeezing through unsized filter cloth under high pressure. The crude turbid juice was cleared as far as possible by centrifuging before being used. The following table gives the figures obtained.

By 'concentration  $\frac{1}{36}$ ' is meant that in each 1 c.c. of the liquid to which the figure refers there were  $\frac{1}{36}$  c.c. of plant juice and  $1 - \frac{1}{36}$  c.c. of fungal extract; and so on.

Column A = extract made by suspending 0.2 gr. *Botrytis* powder in 3 c.c. plant juice.

| Plant.  | Concentration of Plant Juice. |                    |                             |                    |                             |                               | A                           | Remarks.                |
|---|-------------------------------|--------------------|-----------------------------|--------------------|-----------------------------|-------------------------------|-----------------------------|-------------------------|
|   | 0                             | $\frac{1}{36}$     | $\frac{1}{18}$              | $\frac{1}{12}$     | $\frac{1}{6}$               | $\frac{1}{2}$                 |                             |                         |
| <i>Cotyledon rosacea</i> (leaf)               | I                             | $1 - \frac{1}{36}$ | $1 - \frac{1}{18}$          | $1 - \frac{1}{12}$ | $\frac{1}{4}$               | $\frac{1}{10}$                | $\frac{1}{10}$              | Clear.                  |
| Bean (leaf) . . . . .                         | I                             | I                  | $1 (-)$                     | $1 (-)$            | Very variable.              | 0                             | 0                           | Turbid.                 |
| Lemon (fruit) . . . . .                       | I                             | $\frac{1}{36}$     | $\frac{2}{9}$               | $\frac{2}{9}$      | $\frac{1}{3}$               | $\frac{1}{10} - \frac{1}{10}$ | $< \frac{1}{10}$            | Clear. Acidity = 1.1 N. |
| Orange (fruit) . . . . .                      | I                             | $\frac{1}{36}$     | $\frac{1}{2} - \frac{2}{9}$ | $\frac{2}{9}$      | $\frac{1}{3}$               | $\frac{1}{4} - \frac{1}{4}$   | $\frac{1}{4}$               | Clear. Acidity = 0.2 N. |
| Potato (tuber) . . . . .                      | I                             | $1 - \frac{2}{36}$ | $1 - \frac{1}{18}$          | $\frac{2}{9}$      | $\frac{1}{3}$               | $\frac{1}{4}$                 | $\frac{1}{4}$               | Turbid.                 |
| <i>Fegatella</i> (thallus) . . .              | I                             | $1 (-)$            | $1 (-)$                     | $1 (-)$            | $\frac{2}{9}$               | $\frac{1}{4}$                 | $\frac{1}{4}$               | Turbid, mucilaginous.   |
| Cucumber (fruit, middle watery portion) . . . | I                             | $1 (-)$            | $1 (-)$                     | $1 (-)$            | $\frac{1}{4} - \frac{2}{9}$ | $\frac{2}{9} - \frac{1}{3}$   | $\frac{2}{9}$               | Slightly turbid.        |
| Cucumber (basal portion)                      | I                             | $1 (-)$            | $1 (-)$                     | $1 (-)$            | $\frac{2}{9}$               | $\frac{1}{6} - \frac{1}{4}$   | $\frac{1}{4}$               | Slightly turbid.        |
| Apple . . . . .                               | I                             | $1 (-)$            | $1 (-)$                     | $1 (-)$            | $\frac{2}{9}$               | $\frac{1}{4}$                 | $\frac{1}{3} - \frac{1}{4}$ | Clear.                  |

The acidity is only given in a few cases; in the majority of plant juices it cannot be measured by a process of neutralizing on account of the formation of precipitates, and the presence of colour, opalescence, &c.

The above figures can only be interpreted in a very general way. When the plant juice forms a clear solution we must attribute its action on the fungal extract to the chemical substances contained in it. The high degree of retardation produced by the higher concentrations of lemon juice is obviously referable to its high acidity (cf. action of citric acid); in the others we must look upon the effect as being due to the combined action of acids, salts (probably including esters), &c. Where the plant juices are turbid, it is only possible with the data yet to hand to speculate as to the interpretation. Here the fine débris present may disturb the equilibrium of the colloidal enzyme in the solution, and may therefore be expected to increase the retarding effect of the plant juice.

The results with bean juice were very surprising in view of the great susceptibility of that plant, so it was necessary to examine this case in more detail. The expressed sap of bean leaf forms a deep green liquid, highly turbid. A portion of this turbidity can be removed by high-speed centrifuging, as also by passing through filter-paper. After the centrifuged liquid has stood for a short time (an hour) a further sediment can again be obtained by centrifuging, and so on. The crude juice can be driven through a Chamberland filter, and comes through free of turbidity. Soon, however, it is found that centrifuging causes a sediment in the filtered liquid. This process of precipitate formation is no doubt partly one of agglutination; it is also due in part to the oxidase action which takes place at the free surface, and which results in the formation of a black precipitate. In the crude juice, therefore, there is present a copious turbidity which cannot be got rid of by simple filtering. When the juice is boiled a very large precipitate is thrown down, and the juice now remains permanently clear. This boiled juice has a much smaller retarding effect than the crude juice.

Apart from the retarding action of substances contained in the bean extract upon the fungal extract, the former induces a marked change upon the potato discs themselves. This phenomenon has already been alluded to (p. 326, foot-note), but the present constitutes a much more striking manifestation. Discs of potato which have been kept for some time in bean juice possess diminished sensitiveness to the action of the fungal extract. Thus in one case a potato disc which had been kept for twenty-four hours in bean extract was found to be apparently unaffected by freshly prepared *Botrytis* extract after a two days' action, while a similar disc which had been kept for twenty-four hours in water was completely disintegrated by the same extract in sixty minutes. This 'hardening' effect is much greater in the case of the expressed sap of the leaves than in that of the stem; it is also more marked with potato than with turnip

discs. What the explanation of this phenomenon is it is at present impossible to say.

As participating in the abnormal retarding effect of bean juice upon the fungal extract, we have therefore to consider at least three factors:

1. The retarding action (properly so called) of chemical substances present in the bean juice.
2. The effect of the precipitate present.
3. The 'hardening' effect of the bean juice upon the potato tissue employed.

In comparing the experiments *in vitro* with what happens when the fungus invades the bean plant, it is to be noted that factors 2 and 3, which play a considerable part in the former, may not, and probably do not, have their counterpart in the latter.

The experiments above tabulated are very clear on one point—that the expressed sap of *Fegatella* has no special inhibitory action, but behaves in a manner quite comparable with the sap of potato, cucumber, &c. The marked resistance of the tissue of hepatics to the action of the fungal extract and to the fungus itself is not to be ascribed in any way to any specific anti-properties of the cell-sap.

#### H. DISCUSSION OF THE NATURE OF THE 'LETHAL PRINCIPLE'.

It has been shown in the foregoing that the macerating action of the fungal extract can be destroyed in various ways: by heat, by mechanical agitation, and by neutralization with alkali. The extracts so deactivated possess no lethal activity whatever. From microscopical investigation it is known that death of the cells takes place at a late stage in the process of disintegration of the cell-walls. The latter process is therefore the determining factor of the whole action. This dependence of lethal upon macerating activity may be explained in either of the two following ways:<sup>1</sup>

1. That both actions are due to the same substance or group of substances.
2. That the two actions are due to different substances, but the lethal substance is unable to reach the protoplast until the permeability of the cell-wall has been sufficiently increased by the action of the macerating substance.

In the absence of an exact knowledge of the diffusive capacity of the

<sup>1</sup> It might be suggested that no toxin exists in the fungal extract, but that it is produced as a result of the action of the extract upon the cell-wall. Experiment has shown that a fungal extract in which a quantity of well-washed, grated turnip tissue has been digested behaves similarly to standard extract as regards deactivation by heat and by neutralization with alkali. In other words, the hydrolysis products of cell-wall substance are not of toxic nature.

lethal principle in relation to the wall of the cells of susceptible tissue (a knowledge which obviously can be obtained only by indirect means) it is impossible to decide with complete certainty between the two hypotheses presented. Nevertheless from the following experiment the view that the two actions are brought about by the same substance is rendered the more probable.

In Section F it was shown that extracts which had not been completely deactivated by heat possessed lethal activity; and in Section G that both actions were stopped sharply when the extract was neutralized. If therefore the lethal and macerating substances are different, it is improbable that heat and percentage of alkali would affect both in the same degree. Killing of the cells should thus continue independently of the macerating action after a certain stage is reached, that is, when the permeability of the cell-wall has been sufficiently increased. Nevertheless it is found that if the macerating action is stopped, even at a very late stage, the killing effect is strongly retarded.<sup>1</sup> Such evidence is most readily interpreted according to the view that the lethal and macerating substances are identical.

If we accept the hypothesis that lethal and macerating actions are due to the same substance, death of the cells is to be looked upon either as due to the *direct* action of the macerating substance upon the protoplasmic membrane, or as the *indirect* result of the action upon the cell-wall, the phenomenon thus depending upon some special relationship between cell-wall and protoplasm. The former alternative predicates toxicity of the macerating substance; in the latter case death of the cells follows disintegration of the cell-walls in a manner that is not understood.

On the nature of the macerating substance little need be said. The present investigation bears out the conclusions of earlier workers that it is enzymic in nature. In the older literature it was known under the general name of 'cytase'; more lately it has been designated 'pectinase', from its

<sup>1</sup> It is impossible to stop the action of the extract by washing the partially disintegrated discs in water. The active principle remains adsorbed on the tissue, so that discs which have been taken from the active extract even at a comparatively early stage in the action and thoroughly washed in water are completely disintegrated in course of time. It is obvious, therefore, that no conclusions can be drawn from the behaviour of discs which have been taken from active extract and placed in extract which has been deactivated by heat. The only practicable method is to stop the macerating action by immersion of the discs in very dilute alkali ( $\frac{n}{400}$ ), after which they are transferred to an extract which has been rendered exactly neutral. In experiments with discs of Swede Turnip, it was found that up to and a little beyond the stage termed 'coherence gone', the above treatment considerably delayed the incidence of death. As the cell-walls have by this time undergone considerable disintegration, they cannot be conceived to be impermeable to the lethal principle present in the neutralized extract. We should therefore expect that discs at this advanced stage would show the killing effects as rapidly in the neutralized as in the ordinary extract. This, however, is not the case. That the discs in the neutralized extract do show killing after a longer or shorter time (depending on the stage at which they were removed from the active extract) is not surprising. It is probable that a certain amount of action on the protoplasmic membrane had already taken place when the discs were transferred to the neutralized extract.

pronounced action on the pectin constituents of the cell-wall, and more especially on the so-called calcium pectate of the middle lamella.

Whichever hypothesis be accepted as explaining the lethal action of the fungal extract, it is clear in any case that the chemical nature of the cell-wall is of fundamental importance in relation to the action of the fungal extract upon the cell. In all cases it has been found that if the cell-wall is disintegrated death of the cell ensues; if the cell-wall is not affected neither are the living contents of the cell. In other words, the nature of the cell-wall affords the key to the resistance of the particular tissue to the action of the fungal extract and therefore also of the fungus. In particular, certain experiments lead to the conclusion that there are important chemical differences between the cell-walls of higher plants and those of lower forms such as Hepaticae. These considerations point to the desirability of a more complete study of the hemicellulose (or pectin?) series of cell-wall constituents than has yet been attempted.

This investigation was undertaken at the suggestion of Professor V. H. Blackman, and has been prosecuted throughout under his guidance. It is with great pleasure that I take this opportunity of recording my indebtedness to him for many helpful suggestions and for his continued interest.

#### I. SUMMARY.

1. A method of preparing a very powerful extract from the germ tubes of *Botrytis cinerea* is described (Sections C and E, a).
2. The action of the extract on plant tissue is twofold:
  - (a) Action on the cell-wall, leading to disintegration of the tissue.
  - (b) Action on the protoplast, producing death (Section E, b).
3. From microscopical investigation, death of the cells is seen to take place at a late phase of the process of disorganization of the cell-wall (Section E, b).
4. The extract may be deactivated by heating, by mechanical agitation, and by neutralization with alkali. Deactivation by any method leads also to the loss of the lethal power of the extract (Sections F and G).
5. Neither oxalic acid nor oxalates play any part in the toxicity of the extract. If any special lethal substance is present it must be of colloidal nature (Section F).
6. The only active substance in the extract appears to be the enzyme, which produces a macerating action mainly by solution of the middle lamella. The enzyme appears also to be responsible for the lethal action of the extract, the death of the cells being brought about either by direct action of the enzyme on the protoplasmic membrane, or indirectly as a result of the action upon the cell-walls (Section H).
7. The ability of certain tissues to resist the action of the extract is dependent upon the special properties of their cell-walls (Sections E and G).



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## Studies in Permeability.

### I. The Exosmosis of Electrolytes as a Criterion of Antagonistic Ion-Action.

BY

WALTER STILES

AND

INGVAR JØRGENSEN.

With fourteen Figures in the Text.

FOR a considerable time now attention has been directed to the subject of antagonistic ion-action in the absorption of substances by plants, and a number of workers have employed different methods for the investigation of the question. Yet it becomes manifest, on reviewing the literature of the subject (24), that the methods employed are generally long and laborious, while the accuracy or general character of the results obtained is open to much criticism. This is especially true of the most favoured group of methods where the use of water-cultures is involved. The notorious variability of plants growing in water-cultures, which necessitates the determination of the degree of accuracy of the results of the experiments (22), has never been taken into account in these researches, and so the correctness of the results is often very doubtful. The most interesting contribution to the subject from a theoretical point of view is that of Szücs (25), but his method, dependent upon the geotropic reaction of the seedling root and hypocotyl, although ingenious, is very laborious, and, as his results show, is not open to any great accuracy unless so many plants are under observation that the experiments become more laborious still.

Osterhout, in introducing a method from physical chemistry in the use of electrical conductivity properties of plant tissue (15), made the method of attack easier. So far, however, the use of physico-chemical methods has been very limited, and in consequence very few aspects of the problem have been considered, and little analysis of what constitutes antagonism has been attempted.

In this connexion it is of importance to compare the researches on antagonism of those workers who have attempted to obtain quantitative data. Although qualitatively antagonism is generally regarded as the hindrance of the entrance into the plant, or through the plasma membrane, of one ion by another ion of the same sign, yet when quantitative results are attempted it is clear that different workers have been measuring different things. Thus Osterhout (18, 19, 20) attempts to measure antagonism between two metallic ions by finding what strength of solution of salts of the two metals are equally toxic, and then mixing these two solutions in various proportions. If then each ion of either metal was as toxic in the mixed solution as in the pure solution, it should follow that all such mixed solutions would be equally toxic. It is found in certain cases that as a matter of fact this is not so, and that the mixed solutions allow of better growth than the pure solutions. The increase of growth, as compared with the growth in the pure solution, is regarded by Osterhout as a measure of the antagonism, which is greatest for one particular ratio of the antagonizing ions, and becomes less as either of the two ions becomes more concentrated at the expense of the other.

On the other hand, Szűcs and some other writers have kept the quantity of one ion (the poisonous ion) constant, and have added various quantities of the other (the depoisoning ion). If the latter is much less toxic than the former, increase in the depoisoning ion reduces the toxicity of the poisonous one. This action is explained by Szűcs as due to the hindrance of the entrance of one ion owing to the presence of the other. When only one ion is present the whole of the absorbing part of the plasma membrane is available for its passage, but when another ion is present a certain proportion of the absorbing part of the membrane will be used by the second ion. Hence, if this is a relatively harmless ion in comparison with the first, the more of the second ion that is present, the less of the more harmful ion will go in, so that within limits the more of the second ion that is added the greater the depoisoning. Above a certain concentration, however, the depoisoning ion will itself exert an injurious action.

In this way Szűcs explains the antagonistic action of aluminium and copper. Now it will be observed at once that in this way of regarding antagonism, no antagonism is to be expected in the mixtures employed by Osterhout. The antagonism between copper and aluminium on the one hand, and that between 'nutrient' metals on the other, seem therefore to be different phenomena.

It thus seems that although Loeb's idea of antagonism was a great advance, it appears to have produced a mechanical way of regarding as one definite phenomenon all the observed cases, whereas it may be due to different underlying causes in different instances (cf. Hawkins (6)).

For the sake of simplicity we may consider the system with which we have to deal, as consisting of the three following phases :

|                      |   |  |
|----------------------|---|--|
| Exterior<br>solution | Complex of colloidal<br>substances (membrane) | Interior of the cell<br>(crystalloids + colloids). |
|----------------------|---|--|

But the system may be much more complex ; for instance, although earlier the cell-wall was not supposed to be active in permeability phenomena, the recent investigations of Hansteen Cranner (5) suggest that this forms an essential part in the mechanism of exchange between the exterior and interior of the cell.

Now the problems of antagonism and absorption in general resolve themselves into considerations of the kinetics of this system ; but of the condition of equilibrium between any two phases we know very little in any single case.

Osterhout's method of attacking the problems consists in examining tissue of a marine Alga, *Laminaria saccharina*, in various solutions having an electrical conductivity equal to that of sea-water. The conductivity of the tissue he regards as a measure of its permeability (17, 21). But the quantity which Osterhout calls the conductivity of the tissue, is really a complex quantity to which all the three phases mentioned above contribute. Höber (7, 8) has pointed out that conductivity, as measured by Kohlrausch's method, gives no true idea of the ion concentration of such a system, as the inner phase only contributes to a slight extent to the total conductivity. When the conductivity increases in Osterhout's experiments, he considers the increase to be due to the higher penetrating power of the ions owing to a change in permeability, but he neglects the necessary effect of this, the diffusion of electrolytes between the inner and external phases of the system.<sup>1</sup> We have elsewhere (23) pointed out the complex character of plant tissue in regard to electrical conductivity, and that a change of conductivity may be due to a variety of different causes.<sup>2</sup>

Osterhout's method, moreover, in its present form, is applicable to so very few cases, adapted as it is to marine Algae only. In the case of higher plants difficulty arises on account of morphological structure—in many plant organs there are different forms of cells which quite possibly have different permeability properties. Thus in the stem an increase in conductivity of the tissue might be due to an increase in concentration of electrolytes in the vessels and tracheides, and have nothing to do, at any rate directly, with permeability of living cells. Also when fairly uniform

<sup>1</sup> Osterhout has never made clear what he regards as the actual changes taking place in his experiments, but he seems definitely to regard as incorrect the view that it is diffusion phenomena which are the cause of his results.

<sup>2</sup> It is interesting to note that systems of this kind have been examined by a different method by Beutner and Loeb (1, 2, 3, 10, 11, 12) by measuring the electromotive forces manifested. But even if the bearing of this work to our subject is still somewhat obscure, it may prove of importance in investigations of absorption and permeability.

tissue is used, as for example, potato tuber, if the tissue is immersed in a weak solution exosmosis takes place from the living cells, and so reduces the conductivity of the cell-sap, while in a strong solution we may expect absorption. Hence changes in conductivity of living tissue in such a case as this would depend very largely on the concentration of the external solution.

Again, where higher plants are concerned, none of the methods in use have to do with the extremely dilute solutions such as one is concerned with in the actual soil solution absorbed by higher plants. Osterhout has suggested that calcium in the soil is useful to the plant in antagonizing the harmful effect of other salts (16), but, as he himself points out elsewhere (14), below a certain concentration none of the ordinary 'nutrient' ions are poisonous, and in the soil these ions are present in extremely dilute solution (Cameron, 4).

In the present paper an account is given of a contribution towards an analysis of the action of salts on plant cells, in which we deal chiefly with the relations between the external solution and the exosmosis of electrolytes.

We do not intend to apply the results obtained to any interpretation of the functions or the structure of the plasma membrane, but to give an idea of the complexity of the problems involved, and to advocate the use of the methods of physical chemistry.

In using the methods of physical chemistry, one can get a better idea of the kinetics of the actions involved, than in the ordinary methods in general use in botanical and agricultural operations, and there is more hope of getting light shed on the complexity of the processes, but it is most necessary to beware of assuming results mechanically in all cases because a line of argument appears correct in one case. Examples of this we shall present in the part dealing with the results of experiments.

#### METHODS.

We have attempted, in these experiments, to obtain some idea of the relations existing between plant tissue and a solution surrounding it, by examination of the changes in electrical conductivity of the latter. If a substance is absorbed from its solution by plant tissue without producing any change in the plasma membrane, it is to be expected that the electrical conductivity of the solution will be lowered, while if the plasma-membrane is altered by the substance in such a way that its permeability to electrolytes is increased, a more rapid diffusion out from the cells into the solution may be expected, and if the concentration of the external solution is low, compared with that of the electrolytes in the cell-sap, the conductivity may be expected to rise.

The tissue used in these experiments consisted of discs of potato tuber (*Solanum tuberosum*, var. King Edward VII). Potato was selected as it yields a very uniform tissue. Discs were cut having a diameter of a centimetre and a weight of about  $\frac{1}{2}$  grm. Twenty of such discs were washed in distilled water, dried on filter-paper, placed in 100 c.c. of solution in a stoppered bottle, and the electrical conductivity of the solution was measured from time to time by Kohlrausch's method. As different sets of tissue vary somewhat, the experiments with potato were done in duplicate; this gave an approximation to accuracy near enough for our purpose.

In some cases living plants of bean (*Vicia Faba*) were used. They were grown in water-culture for some time in order that plants might be obtained with uninjured roots. Plants with as equal a root development as possible were then selected, and each one placed with its roots immersed in 100 c.c. of solution, and the conductivity of this measured as before. With careful selection of equally developed plants it was found that duplicating the experiments yielded results of sufficient accuracy. The curves which follow were plotted from these results, the increase in the electrical conductivity being taken as ordinates, and the time as abscissae.

The preliminary measurements were made in the Department of Physical Chemistry of this University. We would thank Dr. H. M. Dawson for putting at our disposal the resources of his laboratory.

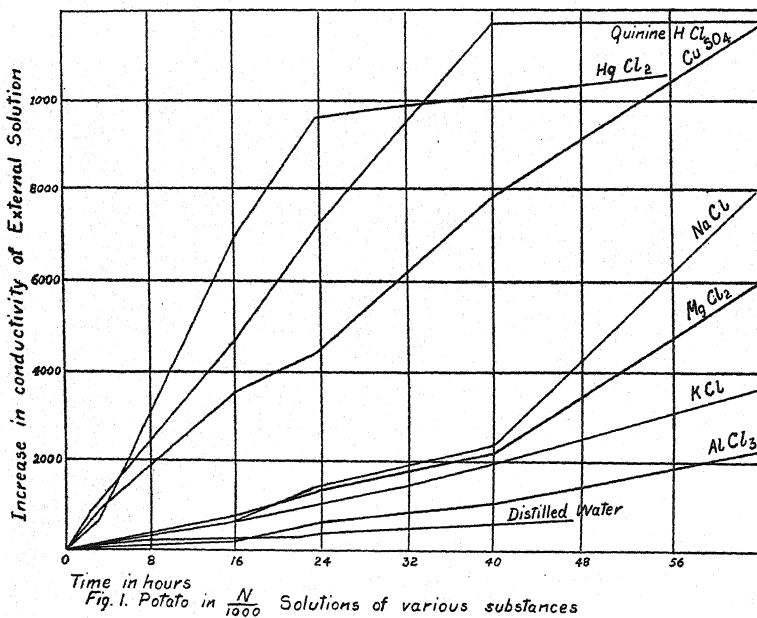
#### EXPERIMENTS.

*Series 1.* In this series various substances were used as external medium in a concentration of  $\frac{N}{1000}$ . The substances employed comprised both undoubted poisonous substances and salts of nutritive metals. The following substances were used:

|                       |   |   |   |   |
|-----------------------|---|---|---|---|
| Copper sulphate       | 'Kahlbaum, with certificate of guarantee' |   |   |   |
| Mercuric chloride     | "   | " | " | " |
| Quinine hydrochloride | 'Kahlbaum'                                |   |   |   |
| Calcium chloride      | 'Kahlbaum, with certificate of guarantee' |   |   |   |
| Sodium chloride       | "   | " | " | " |
| Magnesium chloride    | "   | " | " | " |
| Potassium chloride    | "   | " | " | " |
| Aluminium chloride    | 'Kahlbaum'.                               |   |   |   |

As the accompanying curves show, in all these cases exosmosis of the electrolytes in the cell-sap takes place, as indicated by a very definite rise in the electrical conductivity. This rise is on a much greater scale in the case of undoubted toxic substances, than in the case of salts of nutrient metals. The obvious explanation is that the toxicity of the poisonous

metal ions is due to the formation of substances in the plasma membrane which have much greater permeability than the original compounds they replace, with the result that a much greater rate of exosmosis of electrolytes from the cell takes place. It has to be remembered that what we are measuring is the difference between absorption and excretion. It is not therefore altogether safe to take the increase in conductivity as a measure



of diffusion out from the cell, or as a measure of the toxicity of the surrounding solution.

*Series 2.* In this series the change in the conductivity of the external solutions was investigated for solutions of one substance in different concentrations. Copper sulphate, as in Series 1, was used in the following concentrations:  $\frac{N}{125}$ ;  $\frac{N}{250}$ ;  $\frac{N}{500}$ ;  $\frac{N}{1000}$ ;  $\frac{N}{2000}$ ;  $\frac{N}{5000}$ . The accompanying curves show very clearly how the increase in conductivity, and consequently the increase in the number of ions present, is dependent on the strength of the solution.\* From  $\frac{N}{5000}$  to  $\frac{N}{250}$  there is a definite increase in exosmosis, with increasing concentration of copper sulphate.  $\frac{N}{125}$  gives actually slightly less increase of conductivity than  $\frac{N}{250}$ , although this difference may be within the limits of experimental error.

It will be observed that osmotic pressure alone cannot account for the result, as from this point of view the greatest exosmosis would be expected in the case of the weakest solution.

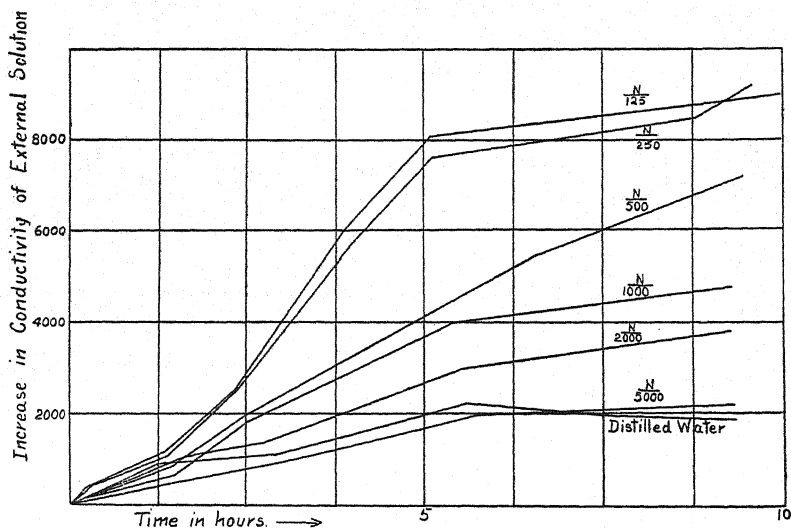


Fig. 2a. Potato in Copper sulphate solutions of various strengths

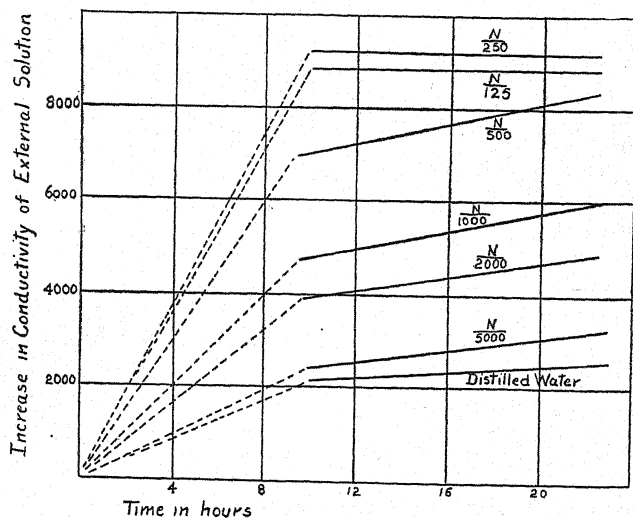


Fig. 2b. The Same curves continued for a longer time

It seems in this case definite enough, that the greater the number of poisonous ions in the solution, the more rapid the exosmosis; or, put in other words, the more rapidly the plasma membrane is rendered more permeable.



*Series 3.* In this series copper sulphate was again used as the external solution, and various strengths between  $\frac{N}{125}$  and  $\frac{N}{5000}$  were employed. Living plants of *Vicia Faba* were used in place of potato as described in the section dealing with methods. It will be observed, by comparing the curves representing the results of the two series, that the bean roots behave in the same way as the potato tissue.

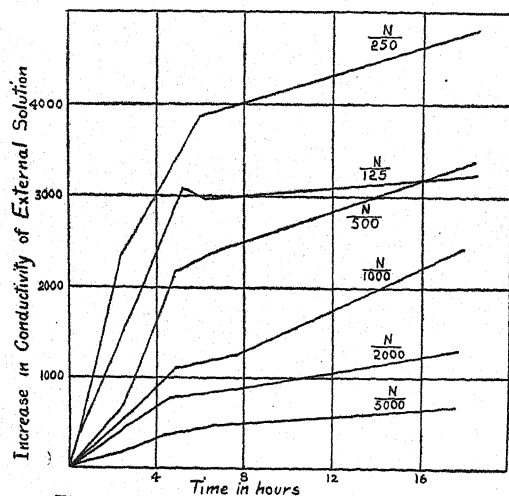


Fig 3 Bean roots in copper sulphate solutions of various strengths

*Series 4.* A series of measurements was made in which potato discs were immersed in various strengths of a solution of lithium chloride. The salt used was manufactured by Merck. The concentrations employed were as follows:  $\frac{N}{125}$ ,  $\frac{N}{250}$ ,  $\frac{N}{500}$ ,  $\frac{N}{1000}$ ,  $\frac{N}{2000}$ ,  $\frac{N}{5000}$ . Although the rise in conductivity of the solution was not so great as in the case when copper sulphate was used, the general result was the same (cf. Fig. 4).

*Series 5.* If the exosmosis of electrolytes from living tissue indicated by the experiments described in the preceding series is caused by the formation from the plasma membrane of substances completely permeable to the electrolytes which diffuse out, it would seem possible that, if the rise of conductivity is regarded as a measure of the extent of exosmosis of electrolytes, we might have here a criterion of antagonism. For if another substance be added to the toxic one, the exosmosis produced by the latter will be reduced if there is any antagonistic action between the two ions, and if the added kation is one which of itself produces little exosmosis, the result should be that in the mixed solution the conductivity will increase at a slower

rate than in the pure solution of the salt, its concentration being the same in the two solutions.

In order to test this possibility various mixtures of lithium chloride with potassium chloride were used. In each case the lithium chloride was

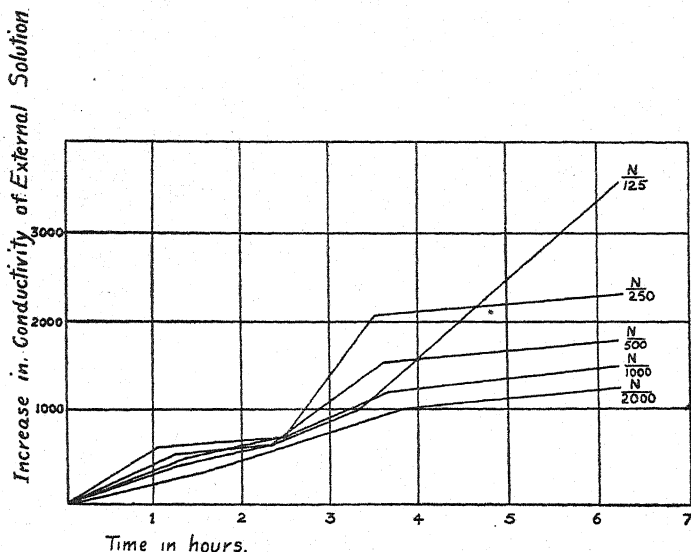


Fig. 4. Potato in solutions of Lithium Chloride of various strengths.

present in the solution in a concentration of  $\frac{N}{2000}$ . Potassium chloride was added to the different solutions so that the following ratios of Li : K were obtained:

I : 0.4

I : I

I : 2

I : 4

I : 7.

The curves in Fig. 5 show very clearly how increasing the quantity of potassium chloride decreases the exosmosis produced by the lithium salt, and this is the more striking as a certain amount of exosmosis does result when potassium chloride is present alone in the solution (cf. Fig. 1). The result can scarcely be due to a depression of the ionization of lithium chloride as a result of addition of potassium chloride. The strongest solution contains a total number of molecules corresponding to no stronger a solution than one of  $\frac{N}{125}$ , and in this dilution nearly the whole of the dissolved substance will be ionized. Also the slowing of the rate of increase

in the conductivity is quite definite in the weakest strength, corresponding to a total concentration of  $\frac{N}{1430}$ . The results obtained do seem to indicate an antagonism between lithium and potassium ions, in the sense that the

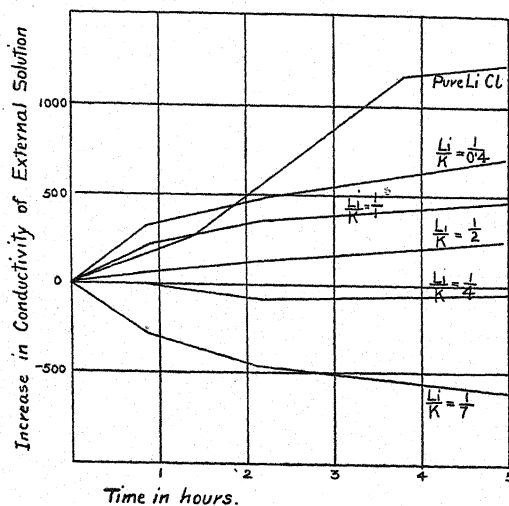


Fig. 5. Potato in  $\frac{N}{2000}$  Li Cl to which various quantities of K Cl have been added.

exosmosis produced by the lithium salt is reduced in the presence of the potassium salt of the same acid.

*Series 6 and 7.* As it has been suggested that antagonistic action is

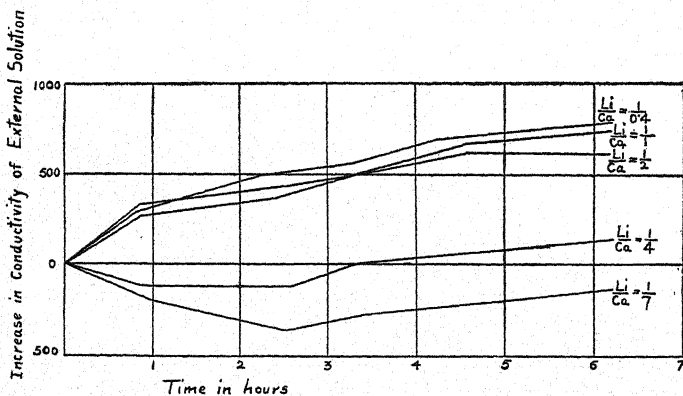


Fig. 6. Potato in solutions of  $\frac{N}{2000}$  Li Cl to which various quantities of Ca Cl<sub>2</sub> have been added. The ratios are given in terms of normal, not molecular quantities.

influenced by the valency of the antagonizing ion (9), similar series were tried in which solutions of lithium chloride of the same strength as that used in the last series were used in conjunction with various amounts of

calcium chloride and aluminium chloride. The kations of both these salts are generally regarded as comparatively harmless ions. The results obtained were similar to those obtained in the mixtures of lithium and potassium chloride, but the results were less rather than more marked. It should be

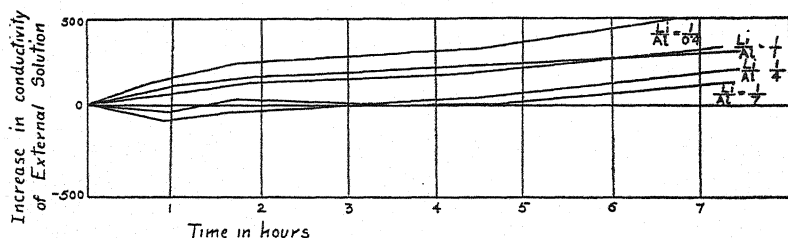


Fig 7. Potato in solutions of  $\frac{N}{1000}$  Li Cl to which have been added various quantities of  $AlCl_3$ . The ratios are given in terms of normal, not molecular solutions

noted that the strengths of calcium and aluminium chlorides are given in normal, not molecular concentration. Practically identical results are obtained in all these cases when molecular ratios are used instead of normal ones.

Series 8–11. The antagonistic action of aluminium chloride on copper sulphate had been made the subject of a very interesting investigation of Szűcs (25). In order to see whether any similar results would be indicated

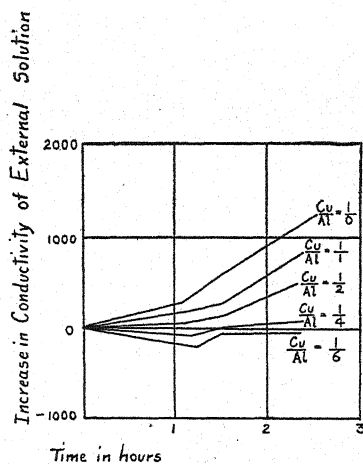


Fig 8. Potato in  $\frac{N}{1000}$   $CuSO_4$  solutions to which various quantities of  $AlCl_3$  have been added

by this electrical conductivity method, the effects of mixtures of copper sulphate  $\frac{N}{1000}$  with different quantities of aluminium chloride were examined with potato tissue. The relative quantities of  $CuSO_4$  to  $AlCl_3$  in the different solutions were 1 : 0, 1 : 1, 1 : 2, 1 : 4, and 1 : 6. During the first

three hours the different solutions behaved very differently, as indicated by the curves in Fig. 8. The more aluminium chloride present, the greater the apparent depression of the exosmosis, so that in the solutions containing most aluminium there is at first a fall in conductivity.

A similar result was obtained when bean roots were used in place of potato (Series 9).

These measurements were repeated several times with a constant result, and the same result was obtained when similar mixtures were made with  $\frac{N}{2000}$  copper sulphate, both when potato (Series 10) and bean roots

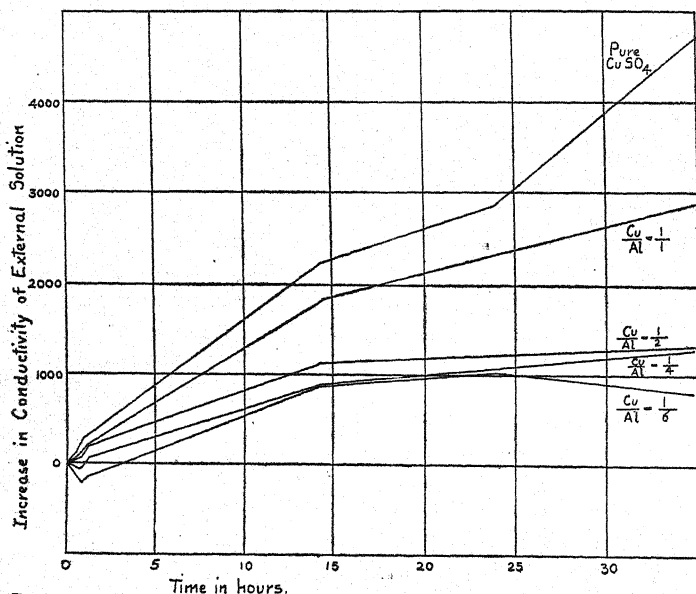


Fig. 9. Bean roots in  $\frac{N}{1000} \text{CuSO}_4$  to which have been added various quantities of  $\text{AlCl}_3$

(Series 11) were used. In the last case the conductivity had risen very little in the mixtures of copper and aluminium, even after forty hours' immersion of the bean roots.

As will be noticed from the curve shown in Fig. 1, aluminium chloride itself produces a little rise in conductivity. Different strengths give little difference in this respect.

At first sight it would appear, then, that by this method a definite antagonism between copper and aluminium is exhibited. This may be the explanation of the results obtained, but, as the next series will indicate, other explanations are possible.

*Series 12.* A series similar to the last was made with potato in copper sulphate  $\frac{N}{2000}$  solution, to which was added various strengths of ferric

chloride (Merck's reagent). In water solution ferric chloride gradually hydrolyses into ferric hydroxide and hydrochloric acid. Controls were therefore used of solutions of the same composition but in which potatoes

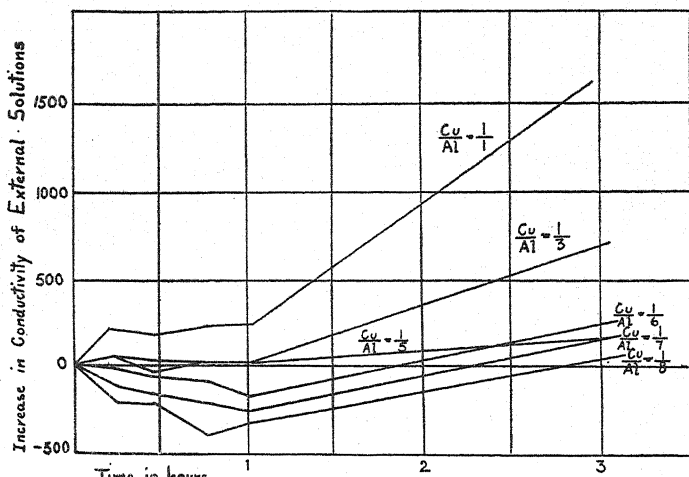


Fig. 10. Potato in Mixtures of  $\frac{1}{2000}$   $\text{CuSO}_4$  with various added amounts of Aluminium Chloride

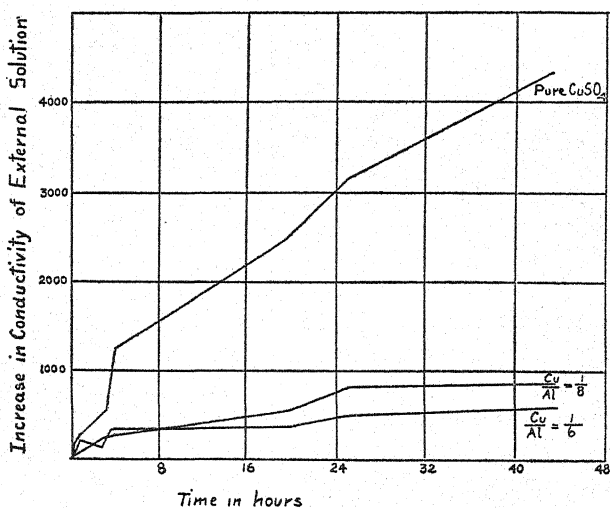


Fig. 11. Bean roots in  $\frac{1}{2000}$   $\text{CuSO}_4$  solution and in mixtures of  $\frac{1}{2000}$   $\text{CuSO}_4$  with  $\text{AlCl}_3$ .

were not placed. Allowing for the rise in conductivity which takes place in these solutions owing no doubt to the production of hydrogen ions, and which is considerable in the solutions containing ferric chloride in greatest amount, the curves representing the results obtained are as shown

in Fig. 12. It will be observed that as ferric chloride is added to the copper sulphate the conductivity of the solution is lowered as in the case of mixtures of copper sulphate and aluminium chloride, but in a very much more marked degree.

A fact which should be mentioned here is the production in the solutions containing plant tissue of an orange-yellow precipitate, presumably of ferric hydroxide, whereas in the control solutions of the same original composition, but without potato, no such precipitation takes place.

From these results one is at once led to inquire whether the formation of hydrochloric acid has anything to do with the form of the curves.

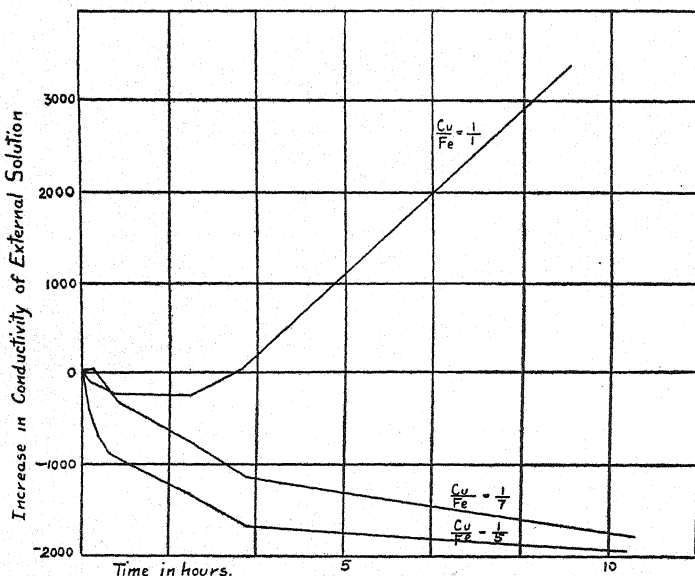


Fig 12 Potato in mixtures of Copper Sulphate and Ferric Chloride

We note a continual decrease in conductivity of the experimental solutions containing much iron, whereas the conductivity of the control increases. The result would be explained if the hydrogen ion were absorbed by the plant almost as fast as it is formed, or at any rate very rapidly.

*Series 13.* In order to test this supposition, solutions of hydrochloric acid were used, potato discs constituting the tissue employed. It was found that a rapid fall in conductivity took place in all the concentrations used ( $\frac{N}{100}$  to  $\frac{N}{16000}$ ). The curve for the case of  $\frac{N}{1000}$  HCl is shown in Fig. 13.

Other acids yield similar results. The rapid fall in conductivity of the solution seems therefore due to the rapid absorption of the hydrogen ion by the plant tissue.

With alkalis a similar phenomenon is observed. Sodium hydrate in various concentrations from  $\frac{N}{250}$  to  $\frac{N}{2000}$  has been used, and a fall in conductivity of the external solution has resulted, as in the case of acids.

In order to obtain more definite information as to absorption of acid by plants we have measured the actual change in the concentration of the hydrogen ion by the use of the hydrogen electrode. It is well known that the contact E.M.F. between a metal and a solution of one of its salts is dependent upon the concentration of the ions of the metal in the solution, and the same applies to hydrogen and acids. A considerable time was spent in trials of numerous forms of hydrogen electrodes. Finally we have adopted the platinum-point form recommended by Michaelis (13) and Walpole (26). This has the advantage of enabling rapid work, and gives all the accuracy necessary for our purpose.

The E.M.F. of the hydrogen-electrode was measured against an  $\frac{N}{10}$  KCl calomel electrode, a solution of 3.5 N KCl being used as intermediate liquid. Kahlbaum's potassium chloride with certificate of guarantee was used, and the mercurous chloride and mercury used for the calomel electrode were specially purified.

By this method of investigation it was found that the hydrogen ion is indeed absorbed rapidly by the tissue. The electrical conductivities of the solutions were also taken, and from the two sets of results it becomes possible to form an idea of the quantity of electrolytes which have diffused out from the plant.

An attempt was also made to determine the change in concentration of the chlorine ion by the use of the electrode chloride solution-calomel-mercury, but the results so obtained are not reliable, for the change in E.M.F. produced by addition of chlorine ions in such an electrode is due to a depression of the number of mercury ions in contact with mercury, and so anything which diffuses out of the plant and can cause a depression of the mercury ions will produce a change in the E.M.F. of the electrode.

The following example will indicate the extent to which the hydrogen ion is absorbed by plants:

Pure  $\frac{N}{1000}$  HCl was used with potato as in the preceding experiments.

The conductivity and concentrations of hydrogen ions of the solutions were measured at various intervals of time. For each pair of measurements two sets of potato discs and solution were used, and conductivity and hydrogen ion concentration of both were measured. The following table gives the results obtained by this means. The results are illustrated graphically in Fig. 13.

We do not propose to give here further data regarding the very impor-



tant question of the permeability of plant tissue to the hydrogen ion, as more detailed investigations of this are at present in progress in this laboratory.

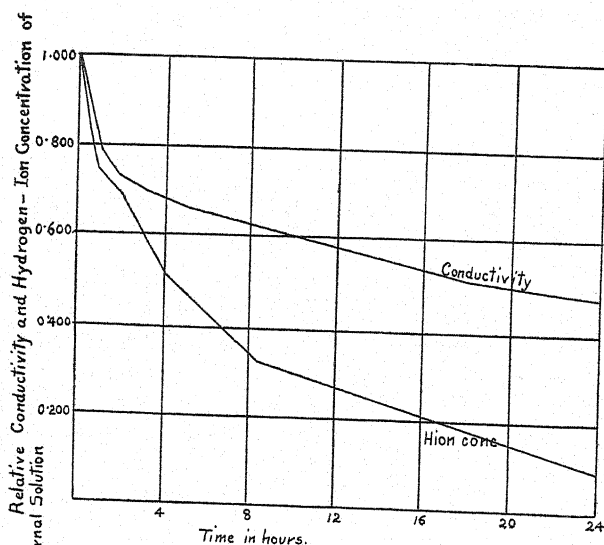


Fig. 13. Absorption of HCl by potato tissue.

#### ABSORPTION OF HYDROGEN IONS BY POTATO TUBER FROM $\frac{N}{1000}$ HCl.

| Time in hours. | Strength of Acid in terms of $\frac{N}{1000}$ HCl. | Conductivity; that of $\frac{N}{1000}$ being taken as unity. |
|----------------|--|--|
| 0.0            | 1.000  | 1.000  |
| 0.5            | 0.821  |  |
| 1.0            | 0.762  |  |
| 2.0            | 0.711  | 0.786  |
| 3.0            |  | 0.742  |
| 4.0            | 0.512  | 0.711  |
| 5.0            |  |  |
| 9.0            | 0.319  | 0.666  |
| 18.0           |  | 0.531  |
| 24.0           | 0.092  | 0.504  |

#### DISCUSSION.

From the results of the experiments recorded above, it seems reasonable to conclude that a diffusion of electrolytes between the inside of plant tissue and the external solution through the cell membranes is a general phenomenon. In our experiments we have dealt mainly with comparatively dilute solutions, and generally here the exosmosis has exceeded the move-

ment of the ions in the reverse direction. When strong solutions are used, an excess of endosmosis is to be expected. This we have observed ourselves with potato tissue when immersed in strong solutions ( $\frac{N}{5}$ ) of sodium chloride and calcium chloride and of mixtures of the two.

Although in dilute solutions of equal strength the relative amount of exosmosis might be regarded as a measure of toxicity, it cannot be assumed that the curves of conductivity of the external solution can so be considered. However suggestive they may be in this respect, it is necessary to remember the complexity of action possible in the solution. The different rates of absorption of different ions and the diffusing out of substances from the plant which react with the external solution are both possible phenomena. As regards the second of these we may cite the case of ferric chloride, in which there results in the external solution conditions which cause the precipitation of ferric hydroxide, while in the control solutions of the same composition no such precipitation takes place. In solutions containing ferric chloride also, the rapid absorption of the hydrogen ions present no doubt influences the conductivity curve exceedingly. It is possible that the same explanation may account for the apparent antagonism between copper and aluminium indicated by the conductivity curves of solutions containing both these ions. No such explanation seems possible of the results in the case of mixtures of lithium with potassium and calcium, but in view of the facts of the case of copper and iron mixtures, we feel that some other reason may account for the form of the curves rather than the apparent one of an antagonism between the two metallic ions.

Nevertheless we feel the results obtained by the method here used suggest a means of obtaining much information relative to the relations between the electrolytes in the cell, the cell membranes, and the external solution. Having regard to the different results obtained in different cases, it is evident that each case will have to be worked out separately. It seems, moreover, quite probable that the different cases of antagonism so far recorded are due in many instances to different underlying causes.

As far as our experiments go, there appears to be no qualitative difference between the behaviour, in respect to permeability to electrolytes, of slices of potato tuber and the roots of uninjured bean plants. This suggests that the methods are suitable for general use, and that general permeability phenomena are being dealt with.

It becomes clear from the experiments here described that the processes involved when plant tissue is immersed in a solution of one salt or a mixture of salts are complex. It thus seems to us that many of the generalizations that have been made by earlier writers, often from single cases, are premature. For example, Osterhout finds that when tissue

of *Laminaria saccharina* is placed in a solution of 0.52 N NaCl, which has a conductivity equal to that of sea-water, the conductivity of the tissue rises with time approximately according to a simple exponential relation. From this he concludes that the change in permeability is due to a change of some substance in the protoplasm, either by a catalytic action of the sodium chloride, or by a reaction with sodium chloride in which very little of the salt is used. He states that a study of the temperature coefficient (a rise of 10° C. more than doubles the rate of reaction) shows that diffusion of sodium chloride inwards and other salts outwards is not the determining cause of the progress of the reaction. But, as our experiments show, with an increase in permeability there is necessarily an increased rate of diffusion through the cell-membrane, which will of itself produce conductivity changes in the tissues. Moreover, when we are dealing with a complex system such as this, the results obtained at different temperatures require careful analysis, before conclusions as to the nature of the processes taking place can be drawn from them.

Because the plant cell is able to absorb inorganic salts Osterhout holds with Loeb and Pauli that the plasma membrane is protein in nature rather than lipid, and that the Quincke-Overton theory is untenable. But while it is now generally realized that the plasma membrane cannot be wholly composed of lipid substances, there is not at present evidence justifying the assumption that it is protein. The cell membrane is one of the most important structures of the cell in regard to its life, and it seems reasonable to suppose that its structure is correspondingly complex. In any case a great deal more experimental work in regard to the permeability of the cell to substances necessary for the ordinary life of the plant is required, before the materials will be obtained for putting forward any satisfactory theory as to the nature of the cell membranes.

#### SUMMARY.

The exosmosis of electrolytes from plant tissue has been examined in relation to the composition of different external solutions by means of physical chemistry methods. Within certain limits it seems reasonable to conclude that the rate of exosmosis is a measure of toxicity. A decrease in this rate when certain ions are added to solutions containing undoubtedly poisonous ions might be due to the same cause that produces what other investigators have called antagonism. In some instances we have shown that the phenomena are more complex than are generally assumed. We have emphasized the necessity of examining and analysing each case separately. The use of the methods of physical chemistry indicate the possibility of obtaining more definite information of the laws governing the exchange of substances between the interior and exterior of the cell.

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# Observations on the Osazone Method of locating Sugars in Plant Tissues.

BY

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With Plate XVII.

IN the course of a research upon the paths of translocation of sugars in plants it has often been necessary to determine the distribution of sugars in the tissues after the plants have been subjected to known external conditions.

The method which has been employed for this purpose is that introduced by Senft,<sup>1</sup> and consists essentially in the production of osazones by means of a glycerine solution of phenylhydrazine acetate, a reagent having good penetrative powers.

Descriptions of the method and of some results obtained by it have already been published,<sup>2</sup> but more recent investigations have thrown additional light on the value of the method and on the limits of its application. For this reason it is considered desirable to give in the present paper a more detailed account of the conclusions arrived at with regard to the use of Senft's reagent and to the interpretation of results yielded by it. In itself the paper forms a necessary prelude to the publication of results obtained in the above-mentioned research.

## GENERAL METHOD.

The reagents employed are phenylhydrazine hydrochloride and sodium acetate dissolved separately in about ten times their weight of *pure* glycerine.<sup>3</sup> Sodium acetate dissolves fairly readily in warm glycerine. The phenylhydrazine hydrochloride should be rubbed up with glycerine in a mortar, warmed and well shaken in a bottle kept stoppered to prevent

<sup>1</sup> Senft ('04).

<sup>2</sup> Mangham ('10, '11).

<sup>3</sup> Commercial glycerine is often adulterated with sugars.

undue access of air. When solution is fairly complete this reagent should be warmed and filtered at least once.

Examined under a microscope, the liquid should be nearly free from brown particles or drops of phenylhydrazine hydrochloride.<sup>1</sup> It should be kept in a darkened bottle preferably having a glass rod attached to the stopper for the withdrawal of drops.

For use, small quantities of the reagents are mixed on a slide, and the plant material is placed in the mixture, covered with a glass slip, and then heated in a water-jacketed oven for any desired time.

The preparation is then ready for examination, and provided that the material is not directly exposed to air by an insufficiency of the reagent, or other cause, nothing more need be done to the slide for some weeks.

It is very important to keep the tissues properly covered with the reagent, as otherwise brown oxidation products form.

Subsequently the sections may be removed, rinsed in cold water or dilute glycerine, and remounted in pure glycerine, the preparation then being sealed with a mixture of gum mastic and paraffin wax applied with a hot wire.<sup>2</sup>

If air bubbles are present in the tissues they can often be removed by an air-pump when the sections are in water or dilute glycerine.

Except when thick the sections generally become moderately clear in pure glycerine. If, however, it is desired to clear them further, weak KOH (2 per cent.) may be employed, after which the material should be well rinsed in water, or water to which a drop or two of acetic acid has been added.

Staining with aqueous stains can to a certain extent be carried out after the above treatment, but this is liable to decrease the transparency of the sections considerably. It is preferable to work with unstained material once familiarity has been gained with the structure of the tissues under investigation.

Strong glycerine produces some plasmolysis, but in practice this does not constitute a serious difficulty.

#### DIFFUSION.

To a certain extent diffusion of cell contents follows the application of the hot reagent, but the amount of this diffusion is less than that which occurs with aqueous reagents such as Fehling's solution.

In a section several cells thick the contents of the more deeply seated

<sup>1</sup> Such syrupy drops, if present in quantity, resemble the osazone syrup given by maltose (see below). Syrup found inside intact cells after treatment with the reagent could, however, hardly consist of small particles of phenylhydrazine hydrochloride, as these would have been filtered out by the cell membranes.

<sup>2</sup> Cf. Thomas ('11).

cells naturally are less liable to diffusion than are the contents of more superficial cells.

Similarly, tissues in which intercellular spaces are rare or absent, e. g. phloem, are less affected by diffusion than are those in which such spaces are large or numerous, e. g. cortex, &c.

Actual observations of numerous preparations give the impression that, after heating for an hour at 98°-100° C., diffusion from cells not injured by the razor is comparatively small, and that the crystals of osazones formed at the surface of the sections and in the medium away from the sections themselves are produced mainly from sugars which have escaped from the cut cells and have diffused into or have become mechanically mixed with the reagent during mounting and subsequent heating.

In support of this contention it may be stated that in numerous instances longitudinal sections of leaf-veins, examined for sugar distribution after the leaves had been darkened for suitable periods, have been found to show a distinct and fairly continuous gradient in the concentration of osazones, which concentration increases towards the proximal end of the vein irrespective of accidental variations in the thickness of the long, hand-cut sections.

Often, too, preparations have been examined in which fine sieve-tubes, arranged in a single layer, have alone formed a thin portion of the section, yet these have been found to be well filled with osazones.

As far then as intact cells are concerned the distribution of the osazones may be held to approximate closely to that of the reacting sugars present at the time of cutting the section.

In other words, positive results may be used fairly safely to locate certain sugars in the tissues, while negative results as a rule should not be attributed to diffusion of sugar present in the cells at the time of mounting.

It is well to note, however, that even when sugars are known to be present negative results may sometimes be given.<sup>1</sup>

#### EFFECTS OF GLYCERINE.

The use of glycerine in the reagent has several advantages. It penetrates rapidly, clears up the section, does not evaporate, is a good mounting medium, and owing to its viscosity diffusion of sugars, &c., is less rapid in it than in water.

On the other hand, it has some effect upon the reaction with sugars, a point which does not appear to have received adequate attention at the hands of other botanical workers or critics who have dealt with it.

Various experiments have been carried out with a view to ascertaining

<sup>1</sup> See below, p. 373.



the effect of glycerine on the production of osazones from the four principal plant sugars, dextrose, levulose, maltose, and cane sugar.

As is well known, aqueous solutions of dextrose and levulose give with phenylhydrazine acetate long, fine, yellow, acicular crystals after a few minutes' heating at 100° C. As a rule levulose deposits crystals before dextrose, the latter coming down on cooling. Microscopically the two osazones are usually indistinguishable. They are both fairly insoluble in cold water, but readily dissolve in alcohol.

Maltose, after being heated for an hour or more, produces a yellow syrup which crystallizes after standing for a longer or shorter period. Frequently these crystals look much like those given by dextrose and levulose, but more often they have a broader and flatter form, much like that of a sword blade, and they may be paler in colour.

Cane sugar, if pure, gives osazones only after becoming hydrolysed by prolonged heating.<sup>1</sup> The resulting crystals are like those of dextrose and levulose.

A glycerine solution of the reagents is, however, used in Senft's method, and it has been found that the glycerine tends to hinder or prevent crystal formation to an extent which varies with the different sugars. This follows from the experiments now to be described.

*Experiment I.* Thirteen pairs of test-tubes were set up. Of these, ten pairs contained small and approximately equal amounts of one or other of the four sugars powdered and moistened. To each was added about 0.75 c.c. of the mixed reagent. The tubes were then completed by adding 2 c.c. of water to one set (*a*), and 2 c.c. of glycerine to the other set (*b*). The other three pairs of tubes contained the reagent together with (*a*) water, and (*b*) glycerine, to serve as controls.

Tubes 1-5, i. e. a control pair + one pair of each kind of sugar, were then heated for 35 minutes at 98° C.

Tubes 6-10, i. e. a similar set, were heated for 60 minutes.

Tubes 11-13, i. e. a control pair + a pair containing cane sugar + a pair containing maltose, were heated for 75 minutes, after which the gas was turned off and the tubes were allowed to cool slowly in the bath.

The tubes were then examined periodically and gave results indicated in the table below.

<sup>1</sup> Neutral aqueous solutions of cane sugar are slowly inverted on boiling. Watts, iv, p. 550.

| <i>Heated 35 mins.</i> | <i>On cooling.</i>   | <i>After 4 days.</i>              | <i>After 5 weeks.</i>   |
|------------------------|----------------------|-----------------------------------|---|
| Cane sugar. (a)        | No change.           | Little change.                    | Brown film at top; liquid slightly turbid.  |
| " " (b)                | Quite clear.         | Quite clear.                      | Top layers (1-2 mm.) slightly turbid. No crystals.                                |
| Maltose. (a)           | No change.           | Little change.                    | Brown film at top; liquid slightly turbid.  |
| " (b)                  | Quite clear.         | Quite clear.                      | Liquid turbid at top only. No crystals.   |
| Dextrose. (a)          | Very little osazone. | Very little osazone.              | Liquid slightly turbid. Only very little osazone.                                 |
| " (b)                  | Quite clear.         | Quite clear.                      | Quite clear, except top layers (1-2 mm.). No osazone crystals visible.            |
| Levulose. (a)          | Abundant osazone.    | Abundant osazone.                 | Liquid slightly turbid. Osazone crystals abundant.                                |
| " (b)                  | Abundant osazone.    | Abundant osazone.                 | Liquid turbid at top only. Osazone crystals abundant.                             |
| Control. (a)           | No change.           | Brown film at top. Liquid cloudy. | Film and general turbidity more marked. Yellow-brown deposition on sides of tube. |
| " (b)                  | Quite clear.         | Quite clear.                      | Quite clear, except for top layer (1-2 mm.), which appeared turbid.               |

| <i>Heated 60 mins.</i> | <i>On cooling.</i>                  | <i>After 4 days.</i>                            | <i>After 5 weeks.</i>   |
|------------------------|-------------------------------------|---|---|
| Cane sugar. (a)        | Little change.                      | Little change.                                  | Film at top; yellow-brown deposition on sides of tube; liquid turbid.   |
| " " (b)                | Quite clear.                        | Quite clear.                                    | Quite clear, except top layer (3 mm.), which appeared turbid.           |
| Maltose. (a)           | Little change.                      | Little change.                                  | } Much the same as with cane sugar.                                     |
| " (b)                  | Quite clear.                        | Quite clear.                                    |   |
| Dextrose. (a)          | Abundant osazone.                   | Abundant osazone.                               | Abundant osazone.   |
| " (b)                  | Quite clear.                        | Quite clear.                                    | Quite clear, except at top.   |
| Levulose. (a)          | Abundant osazone.                   | Abundant osazone.                               | } Abundant osazone.   |
| " (b)                  | Osazone began to form after a time. | Abundant osazone. Crystals smaller than in (a). |   |
| Control. (a)           | Liquid somewhat cloudy.             | Liquid cloudy, with film at top.                | Brown film at top; deposition on sides of tube.                         |
| " (b)                  | Quite clear.                        | Quite clear.                                    | Quite clear, except top layer (3 mm.), which appeared brown and turbid. |

| Heated 75 mins.<br>( <i>etc.</i> ). | On cooling.              | After 4 days.            | After 5 weeks.  |
|-------------------------------------|--------------------------|--------------------------|---|
| Cane sugar. (a)                     | Very slight precipitate. | Very slight precipitate. | Very slight precipitate.  |
| " " (b)                             | Quite clear.             | Quite clear.             | Quite clear, except top layer.  |
| Maltose. (a)                        | Little change.           | Little change.           | Film at top; liquid rather turbid. No osazone crystals.               |
| " (b)                               | Quite clear.             | Quite clear.             | Quite clear, except top layer.  |
| Control. (a)                        | Little change.           | Little change.           | Film at top; liquid turbid; yellow-brown deposition on sides of tube. |
| " (b)                               | Quite clear.             | Quite clear.             | Quite clear, except top layer.  |

Several conclusions may be drawn from the above results, though the experiment was merely a preliminary one and by no means strictly quantitative :

1. In the presence of excess of pure glycerine the formation of crystalline osazones may be hindered or entirely prevented.

2. This effect appears to be more pronounced with maltose and dextrose than with levulose.

3. In the presence of water (in excess) chemical changes occur leading to the formation of brown substances, but glycerine (in excess) tends to prevent or retard these changes. Their first appearance at the surface exposed to air suggests that these changes are oxidation processes. They extend into the pure glycerine mixtures more slowly than into the aqueous ones.

4. The reagent itself in the presence of excess of pure glycerine undergoes no appreciable change even after being heated at about 100° C. for more than an hour; the addition of water, however, brings about the changes referred to in 3.

5. Cane sugar may yield a small quantity of osazone crystals if heated for more than an hour,<sup>1</sup> though excess of glycerine was found to prevent the reaction in this particular instance.

Some quantitative experiments were then made in the following manner :

*Experiment II.* Weighed amounts of the dry, powdered sugars were introduced into glass tubes and just dissolved in a drop or two of water. Carefully mixed reagent was then added in known weight.

The respective tubes contained cane sugar, (1) 1 per cent. (i. e. 0.02 gr. sugar and 2.0 gr. reagent), (2) 10 per cent. (i. e. 0.20 gr. sugar and 2.0 gr. reagent); dextrose, 1 per cent.; levulose, 1 per cent.; and maltose, (1) 1 per cent., (2) 10 per cent.

<sup>1</sup> Cf. below, p. 375.

When prepared the tubes were corked, heated for one hour at 98° C., and examined on cooling and subsequently from time to time.

The results given are shown below:

|                 | <i>On removal.</i>   | <i>After 1 day.</i> | <i>After 3 days.</i>   | <i>After 4 days.</i>                   |
|-----------------|--|---------------------|--|--|
| Cane sugar, 1 % | Clear yellow.  | Slightly turbid.    | Little change. Thin film at top.   | Much the same.                         |
| " " 10 %        | Clear yellow.  | Slightly turbid.    | More turbid.   | Slight deposition of osazone crystals. |
| Dextrose, 1 %   | Clear yellow. Crystals had formed half an hour later.                                | Plenty of crystals. | Abundance of small osazone crystals forming a precipitate at bottom of tube.                         | Much the same.                         |
| Levulose, 1 %   | Practically a solid mass of osazone and glycerine. Tube invertible within 5 minutes. | Much the same.      | Much the same. Crystals, being larger than those given by dextrose, were more uniformly distributed. | Much the same.                         |
| Maltose, 1 %    | Clear yellow (as 1 % cane sugar).  | Slightly turbid.    | Much the same.   | Much the same.                         |
| " 10 %          | Darker yellow, but quite clear.  | Slightly turbid.    | Much the same.   | Much the same.                         |

In order to follow the changes microscopically drops of the mixtures were also mounted on slides having depressions ground in one surface, and the cover-slips were attached with wax mixture.<sup>1</sup>

|                 | <i>After ½ hour.</i>   | <i>After 1 day.</i>          | <i>After 3 days.</i>   | <i>After 4 days.</i>   | <i>After 45 days.</i>   |
|-----------------|--|------------------------------|--|--|---|
| Cane sugar, 1 % | Minute yellow-brown globules.  | Little change.               | Little change.   | Little change.   | No osazone crystals.  |
| " " 10 %        | More numerous globules than in 1 %.  | Moderate number of crystals. | Increase in number of crystals.  | Abundance of crystals though much less than in 1 % dextrose. | Abundant spherical clusters of crystals, dense, almost woolly, outline not sharp. (= 'd.l.' type.) <sup>3</sup> |
| Dextrose, 1 %   | Crystallization set in almost at once. Spherical, rather feathery aggregates of fine, acicular, short crystals. (= 'd' type.) <sup>2</sup> | Little change.               | Plenty of moderately large crystal clusters with great numbers of very much smaller, less dense ones present between them. | Much the same.   | Much the same.  |

<sup>1</sup> Minute crystals may be present in the glycerine, and although these may not be visible to the naked eye, they become obvious under the microscope.

<sup>2</sup> Cf. Pl. XVII, Fig. 2.

<sup>3</sup> Cf. Pl. XVII, Fig. 3.

|               | After $\frac{1}{2}$ hour.  | After 1 day.   | After 3 days.   | After 4 days.   | After 45 days.   |
|---------------|--|----------------|---|---|--|
| Levulose, 1 % | Sheaves of long, fine, acicular crystals formed before mounting. (= 'l' type.) <sup>1</sup>                                    | Little change. | Crystal aggregates very distinct from those of dextrose.  | Much the same.  | Much the same.   |
| Maltose, 1 %  | Minute globules of golden syrup. Liquid as a whole a deeper yellow than in cane sugar or blank tests (i.e. the reagent alone). | No crystals.   | Many drops but no crystals.   | Much the same.  | Much the same.   |
| " 10 %        | More numerous and larger globules of syrup, and deeper colour as a whole than in 1 %.  | No crystals.   | A number of crystal aggregates—coarse sheaves, spheres, and irregular clusters of rather blunt-ended crystals, broader than those of 'd' or 'l'—apparently forming from drops of syrup in some cases. Some finer crystals also present. | More numerous crystals. Quite distinct from the 'd' and 'l' types. The finer crystals, however, resemble individuals of the 'd' clusters. | Still larger number of clusters, some united in chains. Very characteristic. Many of the masses quite opaque. (= 'm' type.) <sup>2</sup> |

Although they do not form a complete series, these experiments bring out several interesting points.

In the case of 1 per cent. mixtures which had been heated for an hour it is seen that levulose and dextrose yielded copious crystalline precipitates. The osazone came down more rapidly with levulose than with dextrose,<sup>3</sup> and in the former consisted of sheaves of long, fine, acicular crystals which contrasted strongly with the more spherical and somewhat feathery clusters of smaller, though acicular crystals given by dextrose.<sup>4</sup>

The cane-sugar mixture underwent little change, though some yellow drops of syrup appeared to have formed.

The maltose mixture turned much yellower, and showed minute droplets of syrupy liquid more conspicuously than did the cane-sugar mixture.

<sup>1</sup> Cf. Pl. XVII, Fig. 1.

<sup>2</sup> Cf. Pl. XVII, Fig. 4.

<sup>3</sup> This difference was observed by Senft. See also below.

<sup>4</sup> It may be remarked that these two types of crystal clusters can hardly be regarded as altogether distinctive characters for dextrose and levulose. In low concentrations the difference is less pronounced and may quite disappear. The two forms are referred to as 'd' (dextrose) and 'l' (levulose) types in the results to be described in a later paper, while 'd.l.' indicates such denser, woolly, spherical clusters as are yielded by cane sugar, and 'm' denotes the maltose type.

The 10 per cent. mixture of cane sugar yielded rather dense, lumpy clusters of crystals. The amount increased for a time, but the final yield was much smaller than that given by either of the 1 per cent. mixtures of the constituent hexoses.

The 10 per cent. mixture of maltose yielded crystals after standing for two or three days. The amount increased for some days, and the crystals, which were quite distinct in form, appeared to arise from the drops of syrupy liquid mentioned above. The presence of finer crystals in smaller quantity rather suggests that a certain amount of the maltose had undergone hydrolysis with the production of dextrose. Glycerine has, indeed, been shown to have a hydrolytic action upon cane sugar and upon maltose.<sup>1</sup>

It is, however, apparent that in the case of the 1 per cent. mixture of cane sugar hydrolysis had not proceeded sufficiently to cause a precipitation of osazones from the resulting invert sugar.

On the other hand, after the 10 per cent. mixture had been heated for an hour, enough of the cane sugar had become hydrolysed to produce a good crop of crystals.

It is clear, then, that attempts to distinguish cane sugar qualitatively in presence of its constituent hexoses by comparing the yields of osazones obtained in duplicate preparations, only one of which has been heated,<sup>2</sup> cannot give trustworthy results, since the formation of a precipitate with cane sugar demands its presence in a relatively high minimum concentration if the duration of heating is not to be prolonged unduly.

Still less reliability attaches to the method as a quantitative one, for from the above examples it is evident that after heating for one hour similar osazone yields would result if each of the pair of preparations contained both dextrose and cane sugar in 1 per cent. strength. Indeed the presence of cane sugar, in addition to the hexose, would not be detected by eye any too readily unless present in several times the above amount.

That Senft was led to attach too much importance to this method of attempting to distinguish cane sugar doubtless arose from using 50 per cent. sugar solutions,<sup>3</sup> and apparently neglecting to check the results so obtained by comparison with those given by weaker solutions more comparable in concentration with the contents of plant cells.

To a certain extent the work of Strakosch,<sup>4</sup> who employed the method in an investigation on the sugars of the beet, is open to criticism on the grounds of unreliable technique, and only those of his conclusions with

<sup>1</sup> Donath ('94). Grafe records ('05, p. 21) that maltose undergoes some hydrolysis after 1-1½ hours heating with Senft's reagent.

<sup>2</sup> Hexoses alone slowly yield osazones in the cold.

<sup>3</sup> Senft, l. c., p. 10.

<sup>4</sup> Strakosch ('07), p. 862. Cf. also Ruhland ('12), pp. 219-22, for criticisms.

regard to cane sugar which were founded on evidence other than that derived in the above manner can be regarded as at all trustworthy.<sup>1</sup>

The identification of maltose by the formation of osazones does not appear to have been investigated by Senft.<sup>2</sup>

Grafe<sup>3</sup> noted and figured the characteristic flat, broad needles of maltose phenylosazone as yielded by Senft's reagent, and such crystals have also been figured by the present writer in an earlier paper.<sup>4</sup>

In the experiments just described, and in others of a similar character, the process of formation of the crystals of maltose phenylosazone was followed under the microscope.

It is slow and changes may take place over many days, though with the stronger solutions, or with more prolonged heating, the time is shortened.

The liquid first changes to a deeper yellow, and small drops looking much like 'golden syrup' appear, the amount of this syrup being roughly proportional to the concentration of maltose present. These drops gradually become confluent to form larger drops, or by partial union they may form irregular chains of globules, or again they may yield masses whose contours show obvious signs of their formation by the coalescence of smaller globules. These larger drops of yellowish-brown to very pale yellow syrup may undergo no further change, or if the concentration is sufficiently high they may begin to show signs of the formation of radially arranged, needle-shaped crystals. Ultimately the larger drops give rise to fairly regular, spherical clusters of rather broad, straight, light yellow needles whose points are usually more or less obtuse. Besides these regular, spherical clusters of radiating crystals other more irregular arrangements are to be found as well as single needles. Frequently a number of crystal clusters form in contact, and become arranged in straight or irregularly curved rows with slight constrictions indicating the component clusters. Often the osazone appears as an almost opaque mass, the crystalline nature of which cannot be made out, or is perhaps only recognizable at portions of the external surface.

Examples of these forms are shown in Pl. XVII, Figs. 4-7.

The formation of crystals of maltose phenylosazone after heating for one hour depends then in part upon the original concentration of the sugar in the glycerine, and it seems to be hindered by the great viscosity of this liquid. In low concentrations the syrup stage of the osazone may not be

<sup>1</sup> Cf. also below, p. 379.

<sup>2</sup> Senft records (l. c., p. 25) that in a few instances, after the preparations had stood for some fourteen days, rosettes and sheaves of yellow crystals formed. He considered that these were probably osazones. Cf. Pl. II, Figs. 8-11, where characteristic crystals of maltose phenylosazone are shown in *Ginkgo*, *Daucus*, and *Elodea*.

<sup>3</sup> Grafe, l. c., Pl. I, Figs. 4 and 5. In the latter crystals are apparently forming from yellow liquid.

<sup>4</sup> Mangham ('11), p. 164, Figs. 3 and 4. See also Plimmer ('10), p. 71, Fig. 14.

passed even after six months, while in higher concentrations crystals may appear in a day or two, or even within a few hours if the heating is more prolonged.

Considerable caution must therefore be exercised in attempting to locate maltose in starch-forming plants by means of Senft's reagent. The formation of drops of syrupy liquid within cells, especially if in any quantity, and if in tissues examined after starch dissolution is known to have occurred, in all probability denotes the presence of maltose, though other possibilities are not altogether excluded.<sup>1</sup>

If actual crystals are formed the osazone can be identified with less uncertainty, though here again it is necessary to bear in mind alternative interpretations.<sup>2</sup>

Here it may be remarked that the failure of Strakosch to detect maltose regularly in the leaves of the beet, and his finding of only small quantities in the petiole,<sup>3</sup> may perhaps have been caused partly by the failure of the osazone to crystallize in glycerine.

The effect of glycerine upon the crystallization of the osazones was further investigated by mixing the latter with glycerine in various proportions, heating the mixtures, and allowing them to cool prior to examining them microscopically.

*Experiment III.* Levulose and dextrose phenylosazones were added to pure glycerine so as to give 0.1 per cent., 1.0 per cent., and 5 per cent. mixtures. The tubes containing these were then heated and shaken until the contents appeared homogeneous. The colour of the resulting liquid varied from straw to dark brown according to the concentration of the osazone.

Drops were mounted immediately after heating and mixing, and were examined microscopically at once.

The results obtained are shown in the table below.

| <i>When examined.</i> | <i>Levulose.</i>   | <i>Dextrose.</i>                                 |
|-----------------------|--|--|
| Just after heating.   | <i>Tubes.</i> 0.1 % Clear straw-coloured liquid.   | <i>Tubes.</i> 0.1 % Clear straw-coloured liquid. |
|                       | 1.0 % Dark-brown liquid depositing crystals on cooling and setting stiffly.              | 1.0 % As with levulose.                          |
|                       | 5.0 % Yellowish-brown solid mass on cooling.   | 5.0 % As with levulose.                          |
|                       | <i>Slides.</i> 0.1 % Showed beginnings of crystal formation within an hour.              | <i>Slides.</i> 0.1 % Much as with levulose.      |
|                       | 1.0 % Deposited crystals within a few minutes of mounting. Well formed after 20 minutes. | 1.0 % Much as with levulose.                     |

<sup>1</sup> See below, p. 387.

<sup>2</sup> See below, p. 387

<sup>3</sup> Strakosch, l. c., p. 863.



| When examined.  | Levulose.   | Dextrose.   |
|-----------------|---|---|
| After 18 hours. | <p><i>Tubes.</i> 0.1 % Quite clear. Straw-coloured.<br/>1.0 % No further change.</p> <p><i>Slides.</i> 0.1 % Good crop of short, small, acicular crystals, mostly separate, but some in sheaves or groups.</p> <p>1.0 % Dense formation of crystal clusters, between which the liquid looked turbid. Crystal clusters pale, almost transparent, and not very clearly defined. Some larger sheaves also present.</p>   | <p><i>Tubes.</i> 0.1 % Quite clear. Straw-coloured.<br/>1.0 % No further change.</p> <p><i>Slides.</i> 0.1 % A distinctly smaller crop of crystals than with 0.1 % levulose. Crystals confined to edges of depression—not in latter itself, as with levulose. Individual crystals indistinguishable from those of levulose.</p> <p>1.0 % Much as with levulose.</p>   |
| After 42 hours. | <p><i>Tube.</i> 0.1 % Still quite clear to naked eye. A drop removed and examined microscopically, however, showed a few small crystal clusters and one or two larger ones. Some almost transparent, flocculent masses or films of irregular outline also microscopically visible.</p> <p><i>Slides.</i> 0.1 % Very good crop of crystals, separate or in sheaves, throughout the liquid.</p> <p>1.0 % Dense mass of spherical crystal clusters. The radiating crystals most clearly defined in central portions of each cluster, while their outer ends could not readily be delimited from those of crystals of adjacent clusters. Optically indistinct—crystals apparently not properly separated as such from matrix.</p> | <p><i>Tube.</i> 0.1 % Still quite clear to the eye. A drop examined microscopically showed distinctly fewer crystals than with 0.1 % levulose. Flocculent masses as with levulose—possibly osazone about to crystallize.</p> <p><i>Slides.</i> 0.1 % An increased number of crystals present practically all round edge of depression, but not yet in latter. Similar in appearance to levulose, but much less in quantity.</p> <p>1.0 % Much as with levulose.</p> |
| After 4 days.   | <p><i>Tube.</i> 0.1 % Precipitation of crystals commencing in upper portion of liquid.</p> <p><i>Slides.</i> Much as before.</p>  | <p><i>Tube.</i> 0.1 % Smaller amount of crystals in course of precipitation than in levulose.</p> <p><i>Slides.</i> Much as before.</p>   |
| After 6 days.   | <p><i>Tube.</i> 0.1 % The crystals viewed from above end of tube showed a radial distribution.</p> <p><i>Slides.</i> Much as before.</p>  | <p><i>Tube.</i> 0.1 % As with levulose, but in smaller amount.</p> <p><i>Slides.</i> Much as before.</p>  |
| After 21 days.  | <p><i>Tube.</i> 0.1 % Increased crop of crystals.</p>   | <p><i>Tube.</i> 0.1 % Much smaller crop of crystals than with levulose.</p>   |

| When examined. | Levulose. |   | Dextrose.              |   |
|----------------|-----------|---|------------------------|---|
| After 47 days. | Slides.   | Much the same.  | Slide. 1.0 %           | Crystals more clearly defined.  |
| After 57 days. | Slides.   | Little further change. Crystal formation did not appear to have progressed as with dextrose. Very hazy and turbid or granular, except at centres of clusters. | Slides. 0.1 %<br>1.0 % | Little change. Crystal formation apparently complete. Innumerable very minute crystals everywhere, besides larger clusters. |

From these results it would appear that the osazones are fairly soluble in hot glycerine, but that on cooling they come down as crystals readily in mixtures of 1 per cent. concentration and above, but less readily in 0.1 per cent. mixtures.

In the 1 per cent. mixtures the actual crystal clusters of levulose were ultimately less sharply defined than those of dextrose in this particular instance.

It is also seen that a 0.1 per cent. mixture of levulose phenylosazone deposits crystals more rapidly and copiously than a corresponding mixture of the osazone yielded by dextrose.

The two osazones are almost insoluble in glycerine,<sup>1</sup> but in weak concentrations crystallization seems to be retarded by the viscosity of the glycerine. Naturally in a viscous medium the rate of diffusion of particles to form crystals is slower than in a medium such as water. Clearly, too, the process of crystallization will be slower when the particles are highly dispersed throughout the medium than when they are present in greater concentration.

On the whole, then, although there may be some irregularity at times, it may be held that if the preparations are allowed due time for equilibrium to be attained, the use of glycerine in the reagent as it is ordinarily employed has no very serious effect upon the delicacy of the test as far as levulose is concerned. In the case of dextrose, however, it may occasionally happen that crystals will fail to appear.<sup>2</sup>

The presence of other sugars may affect the reaction given by any particular sugar, but before dealing with this point the results may be described which were obtained by mixing maltose phenylosazone with glycerine and treating the mixtures as in the above experiment.

*Experiment IV.* Tubes were prepared containing the osazone mixed

<sup>1</sup> The actual solubility has not been determined, but it is obviously less than 1 in 1,000.

<sup>2</sup> Grafe states (l. c., p. 17) that a 0.015 per cent. solution of dextrose yields positive results. It is not clear, however, whether this concentration means 15 parts in 100,000 of the reagent or of water previous to the addition of reagent.

with pure glycerine in ten different proportions, viz. 1 per cent., 2 per cent., 3 per cent., 4 per cent., 5 per cent., 6 per cent., 8 per cent., 10 per cent., 15 per cent., and 20 per cent.

As in previous experiments drops of the mixtures were mounted on slides, sealed and examined microscopically at intervals over a long period.

The behaviour of maltose phenylosazone under these conditions differed markedly from that of the osazones of dextrose and levulose.

No rapid re-crystallization occurred, but the preparations showed a series of changes which took place with extreme slowness.

After the tubes had cooled, their contents ranged in colour from pale straw to very deep brown.<sup>1</sup> With the rise in concentration of the osazone the viscosity of the mixture increased, the 20 per cent. mixture being almost invertible.<sup>2</sup> Owing to the opacity of the higher mixtures satisfactory observations could not be made on them in the tubes.

In all the less opaque tubes a slight increase in turbidity was noticed for a few days from the commencement of the experiment.

After four days the tubes up to 6 per cent. showed a somewhat less opaque top layer.

Observations made upon the prepared slides showed that suspended in the fluid medium were numerous very minute globules of yellowish-brown syrup. In any one slide these globules varied in size over a not very wide range. Their number increased more or less in proportion to the concentration of the osazone.

On examining the preparations by dark-ground illumination and with a Zeiss 16 mm. objective and  $\times 18$  compensating ocular the presence of an immense number of minute particles could be detected. These were best seen in the weaker mixtures, and according to their size they scattered rays of different wave length, and so appeared as bright red, orange, green, &c., points of light.

The number of points of light which could be detected by dark-ground illumination was much greater than that of the globules visible by weak transmitted light.

It was possible to observe an extremely slow motion of the points of light in the glycerine, i.e. slow, compared with the motion of colloidal particles in a metallic hydrosol.

The minute yellowish-brown globules showed a fortuitous arrangement at first, but gradually, and more obviously in the higher concentrations, they became grouped to form irregular clusters leaving spaces more or less free. Many of the globules cohered, and some by coalescence gave rise to larger ones. By the fourth day this grouping of globules into chains, &c.,

<sup>1</sup> Probably due to small amounts of aniline or decomposition products present as impurities.

<sup>2</sup> Cf. the hexoses, pp. 379-80. It is possible to prepare emulsions of oil and soap having a consistency allowing of cubes being cut out of them. Hatschek ('13), p. 22.

had become quite evident and numbers of large globules were to be seen surrounded by smaller ones just in contact with them.

Besides this gradual grouping and coalescence of yellowish-brown globules another change soon became evident. When weak light was used a pale yellow substance in the form of more or less regular spheres, often with somewhat darker centres, appeared on the lower surfaces of the cover-slips.

This change was first noticed in the stronger mixtures, but later on it appeared in the weaker ones also. It was observed on about the third day in the 8 per cent. mixture and in those of higher concentration, on the sixth day in the 6 per cent. mixture, the twelfth day in the 5 per cent. mixture, and some days later in the 4 per cent. mixture. Below this strength the pale yellow substance had not appeared after nine weeks from the beginning of the observations, although it was then very noticeable in all concentrations above 3 per cent. At the end of this period the first three slides showed only globules of brownish syrup of various sizes irregularly grouped as described above. (Cf. Pl. XVII, Fig. 8.)

The actual nature of these globules has not been determined, but it is considered probable that they were due partly to impurities, either introduced with the osazone or resulting from decomposition produced by local overheating, and partly to osazone which had been melted,<sup>1</sup> but had not been brought by the heating into a state of fine division, and so had yielded visible droplets of syrup.

At the same time in the 4 per cent. mixture the pale yellow substance took the form of numerous approximately regular spheres having a diameter of the order of ten times that of the average globule of brownish syrup, of which numbers were also present.

Most of these spheres were single, but in some cases two or more appeared fused and occasionally rows of them were formed.

About five weeks later the 3 per cent. mixture showed a small number of these pale yellow spheres. (Cf. Pl. XVII, Fig. 9.) After four months from the commencement of the observations the number of spheres had increased, but none were visible in the 2 per cent. mixture. (Cf. Pl. XVII, Fig. 8.)

In the higher concentrations the amount of the light yellow substance was proportionately greater. While in the 8 per cent. mixture the spheres were for the most part just in contact with one another, above this strength their closeness and fusion caused the preparations to appear opaque and coarsely granular.

It has not been possible to make out the exact structure of these spheres. In many cases they resembled drops of pale yellow liquid, while in a few instances the slightly roughened outline and somewhat granular

<sup>1</sup> Melting-point of maltose phenylosazone, 206° C. Boiling-point of glycerine, 290° C. The osazone is liable to decompose in air at melting-point; cf. Armstrong ('12), p. 60.

appearance recalled some stages observed in the formation of osazone crystals in preparations in which maltose had been heated with the reagent.

On the whole it seemed fair to conclude that the osazone, which as the result of being heated had melted and had then been dispersed throughout the glycerine as an emulsion and partly as a solution, on cooling had gradually become aggregated into microscopically visible droplets which were in some cases very slowly undergoing re-crystallization from the syrupy condition.

Although after six months from the time of mounting some of these spheres appeared distinctly more crystalline, the majority were still almost transparent and structureless.

In addition to these observations, others were made on preparations which contained drops of the 1 per cent. and 5 per cent. mixtures described above, and which had been heated at 98° C. or so for  $\frac{1}{2}$  hour, 1 hour,  $1\frac{1}{2}$  hours, and 2 hours respectively after having been set up.

The early stages of the processes just dealt with were somewhat accelerated by the heating. About four months later it was found that, while the 1 per cent. mixtures which had been heated for 1 hour and  $1\frac{1}{2}$  hours respectively showed only drops of yellowish-brown syrup, there also appeared in the other two 1 per cent. preparations a great number of much larger, pale yellow, spherical, semi-crystalline or granular masses. (Cf. Pl. XVII, Fig. 10.)

These evidently consisted of osazone partially re-crystallized, and so afforded an example of a later stage in the process than could be seen in any of the unheated slides. After six months from the beginning of the experiment the osazone in all of these slides was found to have partially re-crystallized.

It is clear, therefore, that even in 1 per cent. concentration the viscous syrup may under suitable conditions slowly crystallize.

Crystallization is, however, uncertain and the osazone may remain either in solution or as a fine emulsion, or it may separate out as microscopically visible drops of syrup which apparently do not crystallize.

Doubtless in this case, as with other organic compounds, the presence of impurities hinders crystallization of the syrup. It is known that in aqueous solutions the form of the crystals of maltose phenylosazone is greatly affected by small traces of impurities.<sup>1</sup> In the plant cell many substances are present and must constitute impurities; among these are colloids, the influence of which on crystallization may be very marked.

Accordingly some lack of uniformity of the osazone of maltose is to be expected when it is formed inside vegetable cells, and especially inside sieve-tubes where proteins may abound.

It cannot, of course be taken for granted that results yielded by extra-

<sup>1</sup> Armstrong, l. c., p. 60.

cellular experiments will hold good for reactions carried out inside plant cells, for it is impossible to realize the precise conditions obtaining in the latter. Still it may be urged that the above results and considerations afford some justification for regarding as maltose phenylosazone the yellow syrup so often observed inside cells (especially the sieve-tubes of fine veins in leaves) after treating with Senft's reagent sections of veins, petioles, &c., taken from starch-forming leaves which had previously been placed under conditions ensuring hydrolysis of starch and translocation of sugar.

Furthermore, it may be suggested that the production of an apparently homogeneous syrup in these cells probably indicates the presence of maltose alone,<sup>1</sup> while the appearance which has been recorded in notes of experiments as 'semi-crystalline', 'amorphous', or 'granular', and which has been found on the whole less commonly in the sieve-tubes of the finer veins of leaves than in those of the stronger ones, would then denote either maltose<sup>2</sup> from which osazone crystals had formed (or were forming) and were mingled with some uncrystallized syrup, or a mixture of maltose with other sugars.

For example, such an appearance might well be produced if maltose, in the course of translocation, gradually became hydrolysed under the action of maltase produced by the sieve-tubes themselves or by the companion-cells,<sup>3</sup> or if hydrolysis occurred during the heating of the preparations.<sup>4</sup> The resulting dextrose would yield osazone crystals, but there would probably be some syrup as well if any maltose remained, and in the confined space of the sieve-tubes the two would be obliged to mix to a certain extent.

Again, if in addition to maltose cane sugar had entered the sieve-tubes, and sufficient of it had become hydrolysed either during the heating or previously while undergoing translocation, a deposit of crystalline osazone might be formed and so produce the appearance referred to.

Finally, there might also be present with the maltose before treatment with the reagent hexoses which had not arisen from the hydrolysis of bioses.<sup>5</sup>

#### REACTION WITH MIXTURES OF SUGARS.

It was remarked above that the presence of other sugars may influence the osazone reaction given by any particular sugar.

In this connexion considerable interest attaches to the work of Scherman and Williams,<sup>6</sup> who studied the rate of precipitation of osazones from aqueous solutions of one or more sugars. Other conditions being unchanged, they found that the rate of precipitation with dextrose varied with

<sup>1</sup> Probably in low concentration.

<sup>2</sup> Probably in higher concentration.

<sup>3</sup> Cf. Scott ('89), p. 156.

<sup>4</sup> Cf. above, p. 377.

<sup>5</sup> In this connexion cf. Brown and Morris ('93), Strakosch, l. c., Parkin ('11), Ruhland, l. c., Campbell ('12), and Armstrong, l. c.

<sup>6</sup> Scherman and Williams ('06).

the concentration of the solution and was approximately constant for any given dilution.

Similar results were obtained with levulose, which, however, always gave a precipitate in about one-third of the time required by the same amount of dextrose.

Invert sugar yielded the osazone almost as rapidly as levulose of the same concentration.

The rate of precipitation was accelerated by the addition of certain other sugars. For example, the time required for precipitating the osazone from a solution containing 0.1 per cent. of dextrose was shortened considerably in the presence of 5 per cent. raffinose, a sugar giving no direct reaction itself.

Cane sugar was found to produce a similar acceleration with levulose solutions.

On the other hand, maltose, and to a greater extent lactose, retarded the precipitation and interfered much more seriously in the case of dextrose than in the case of levulose.

These results obtained in aqueous solutions have not been compared fully with those given in glycerine solutions. But some of the earlier experiments carried out by the author before becoming acquainted with the above work showed that mixtures of dextrose and maltose gave the reaction less readily than mixtures of levulose and maltose, and that in a mixture of dextrose and levulose the reaction did not appear to be at all hindered.<sup>1</sup> Moreover, in the case of the levulose and maltose mixture typical levulose osazone crystals were found on examining immediately after the heating, whereas no crystals could be seen in the dextrose and maltose mixture until about two hours later, and then small ones slowly formed. A similar dextrose solution alone gave large crystals immediately on cooling. In these experiments the preparations were heated for half an hour.

As the behaviour of dextrose and levulose in glycerine is on the whole much the same as in water, there is little reason for doubting that the results obtained in aqueous solutions would apply also when the reaction is carried out with Senft's reagent, although the effect would be intensified owing to the viscosity of the glycerine.

Here then is another reason for allowing due time to elapse before drawing conclusions from the application of the reagent to plant tissues known to form starch and thus likely to contain maltose and dextrose.

Various points in connexion with the use of Senft's reagent in botanical work have now been dealt with, but one or two more call for remarks.

*Oxidation.* The formation of brown substances in presence of air, and more rapidly when water is present too, has been referred to above. This

<sup>1</sup> These experiments were only roughly quantitative, as drops of 5 per cent. aqueous solutions were added to Senft's reagent in approximately equal amounts in the various cases.

is probably due to the oxidation of the reagent, for phenylhydrazine has not good keeping properties when freely exposed to the air. Pure glycerine acts as a preservative, presumably because it prevents ready access of air. The products formed give in glycerine brown drops of syrup (cf. foot-note below).

The glycerine itself might conceivably undergo oxidation<sup>1</sup> by the action of substances present locally in the plant, in which case small quantities of such compounds as glyceric aldehyde or dihydroxyacetone might be formed, and these substances yield osazones. To examine this point some glycerine was oxidized with aqueous ferrous sulphate and hydrogen peroxide,<sup>2</sup> and the brown substance (osazone) resulting from the subsequent addition of Senft's reagent was examined microscopically. The (dilute) glycerine mixture yielded drops of brown syrupy liquid, some opaque brown spherical masses, and some crystal clusters, the individual crystals of which had the form of lamellae almost as broad as long and with obtuse ends. To some extent such crystals resemble those yielded by maltose phenylosazone, and might possibly be mistaken for them if not closely examined. The syrup is practically indistinguishable from that of the maltose osazone, although rather darker in colour.

It should be noted that the reagent itself, when tested in blank experiments used as controls to those in which sugar had been added, did not give crystals; if properly filtered in preparation very little syrup is formed either.<sup>3</sup>

On the whole the danger of mistaking for crystals of maltose phenylosazone those of the osazone of an oxidation product of glycerine is small under the conditions in which the test is ordinarily applied.

*Salts of Phenylhydrazine.* Phenylhydrazine readily forms salts with acids, and some of these are insoluble in glycerine and water. The hydrochloride is fairly soluble in glycerine, the acetate also, but the oxalate is less soluble. The crystals, however, are in each case quite distinct in form from those of maltose phenylosazone. Moreover they are typically colourless, but might easily appear yellowish in the proximity of yellow osazones. While in general these crystals are distinguishable still in some arrangements, especially when viewed edgewise, and when at all inclined to appear yellow, careful examination may be necessary to determine their real identity.

<sup>1</sup> In contact with platinum black glycerine in presence of air and water produces glyceric aldehyde, CO<sub>2</sub> and water. Watts, ii, p. 616. In contact with iron oxidizing in moist air, a substance like glucose is formed. Ibid., p. 617.

<sup>2</sup> Fenton ('06), p. 101.

<sup>3</sup> Cf. p. 370, foot-note 1. If, however, the reagent is old when applied, or is heated for a long time in presence of air, yellowish-brown liquid and crystalline or opaque masses may be formed after standing for some months. It is advisable to renew the phenylhydrazine hydrochloride solution at least once every six months, though Senft states (l. c., p. 7) that the reagent may be used satisfactorily even if three years old.



## SUMMARY.

The results of this investigation may now be brought together.

To carry out the test sections of plant tissues are laid in a mixture of glycerine solutions of phenylhydrazine hydrochloride and of sodium acetate, and then are heated at  $98^{\circ}$ – $100^{\circ}$  C., usually for an hour.

During the heating to which the tissues are subjected some diffusion of cell contents may occur, but this is certainly less than that resulting from the use of aqueous reagents such as Fehling's solution. On the whole positive results (osazone formation) may be held to indicate with a fair degree of accuracy the distribution of the reacting sugars before treatment with the reagent.

The reaction is affected by the glycerine employed; the glycerine acts mainly by reason of its viscosity and causes a retardation of processes depending upon diffusion.

The amount of this retardation varies with different sugars, and apparently is not altogether constant for the same sugar.

Levulose yields an osazone very readily, and in preparations heated for half an hour the crystals are often formed before cooling. Frequently the crystals are long and fine, and are arranged in sheaves.

Dextrose precipitates its osazone less readily than levulose, and with very small concentrations of dextrose a positive result may occasionally not be given. As a rule the crystals are shorter, and are formed in spherical clusters having a feathery outline. With 1 per cent. of the sugars present the crystal clusters contrast strongly in the two hexoses, but in low concentrations this difference disappears. With 0.1 per cent. of the osazones present the crystals are small and indistinguishable, and are deposited after a few hours, but more readily and copiously in the case of levulose.

Too much reliance should not be placed on the crystal cluster form as a feature distinguishing dextrose from levulose.

Glycerine is known to have a hydrolytic effect on cane sugar and maltose.

Cane sugar, if present in sufficient concentration, may become partly hydrolysed after being heated at  $98^{\circ}$ – $100^{\circ}$  C. for an hour. In an experiment with 1 per cent. of the sugar present no osazone crystals were formed. With 10 per cent. a good crop of dense, lumpy crystal clusters was obtained, but the yield was much less than that given by a 1 per cent. dextrose mixture.

It follows that attempts to detect cane sugar in presence of its constituent hexoses by comparing the osazone yield in duplicate preparations, one of which only has been heated (hexoses alone react slowly in the cold), cannot give very reliable results and may be quite misleading.

The presence of water appears to accelerate the hydrolysis of cane

sugar, so that as a little water is present in tissues at the commencement of heating, it is quite possible that the reagent is more sensitive to cane sugar in actual practice than the above experiments would suggest. Moreover, an acid cell-sap, &c., would assist in the hydrolysis of the cane sugar.

Maltose, after being heated for an hour, forms a golden yellow syrup from which crystals may slowly form. In a mixture containing 1 per cent. of sugar only the syrup was produced, and this appeared in the form of a coarse emulsion; but with a 10 per cent. mixture, after a day or two, there resulted a small crop of fairly large, straight, linear-lanceolate, obtuse-ended crystals arranged either radially in spherical clusters or in various irregular groupings. The yield increased slowly over a number of days. Many of the clusters were almost opaque, and their crystalline nature could only with difficulty be made out.

The presence of impurities in the form of various cell contents, particularly colloidal substances, probably influences the crystallization of the syrupy osazone, and may account for some irregularity in its behaviour in plant tissues.

From observations on the process of crystallization of maltose phenyl-osazone in glycerine, it is concluded that the production of a golden yellow syrup inside starch-forming cells, or conducting cells of starch-forming organs, which have previously been treated with Senft's reagent, very probably indicates the presence of maltose.

It is suggested that a commonly observed granular appearance of this liquid may be due to one or more of the following causes:

- (a) Crystallization of syrupy maltose phenylosazone;
- (b) Partial hydrolysis of maltose originally present with production of dextrose and its osazone; this may occur during the heating in Senft's reagent, or the maltose may have been undergoing enzyme hydrolysis at the time of applying the reagent;
- (c) Similar hydrolysis of cane sugar present with maltose, and consequent production of invert sugar yielding osazones;
- (d) Presence of maltose, together with hexoses not produced by hydrolysis of disaccharides, i. e. 'up-grade' hexoses.

Maltose appears to retard precipitation of osazone crystals of the hexoses; this effect is more marked with dextrose than with levulose.

The reagent itself gives no crystals after two or three hours' heating, and if properly filtered in preparation only small traces of syrup are formed. If old, however, or much exposed to air, the reagent alone after heating for an hour or so may yield crystalline compounds and syrup after some months standing. This does not occur in properly closed preparations in which moderately fresh reagent has been used. The presence of water assists in the formation of these products. It is as well to renew the phenylhydrazine hydrochloride solution at least once every six months.

There is a slight possibility of mistaking for the osazone of maltose other crystals such as those of salts of phenylhydrazine, or of the oxidation products of glycerine should these be formed.

In conclusion, it may be said that when using Senft's reagent it is advisable to re-examine the preparations from time to time over a period of at least four months before attempting to draw conclusions from them.

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#### DESCRIPTION OF PHOTOMICROGRAPHS IN PLATE XVII.

Illustrating Mr. Mangham's paper on the Osazone Method of locating Sugars in Plant Tissues.

Fig. 1. Osazone crystals yielded by 1 per cent. of levulose in Senft's reagent after heating for one hour. Photographed four months after heating.  $\times 114$ .

Fig. 2. Osazone crystals yielded by 1 per cent. of dextrose in Senft's reagent after heating for one hour. Photographed four months after heating. Note feathery edges of crystal clusters.  $\times 114$ .

Fig. 3. Osazone crystals yielded by 10 per cent. of cane sugar in Senft's reagent after heating for one hour. Photographed four months after heating.  $\times 114$ .

Fig. 4. Osazone yielded by 10 per cent. of maltose in Senft's reagent after heating for one hour. Photographed four months after heating. Many of the masses were almost opaque.  $\times 114$ .

Note.—Figs. 1–4 are not intended to indicate the relative amounts of osazone produced, but to illustrate the types of crystal aggregates formed.

Fig. 5. Drops of syrup formed after maltose had been heated with Senft's reagent. Photographed one month after heating.  $\times$  *circa* 120.

Fig. 6. Stages in the formation of crystals of maltose phenylosazone from yellow syrup. Photographed one month after heating.  $\times$  *circa* 120.

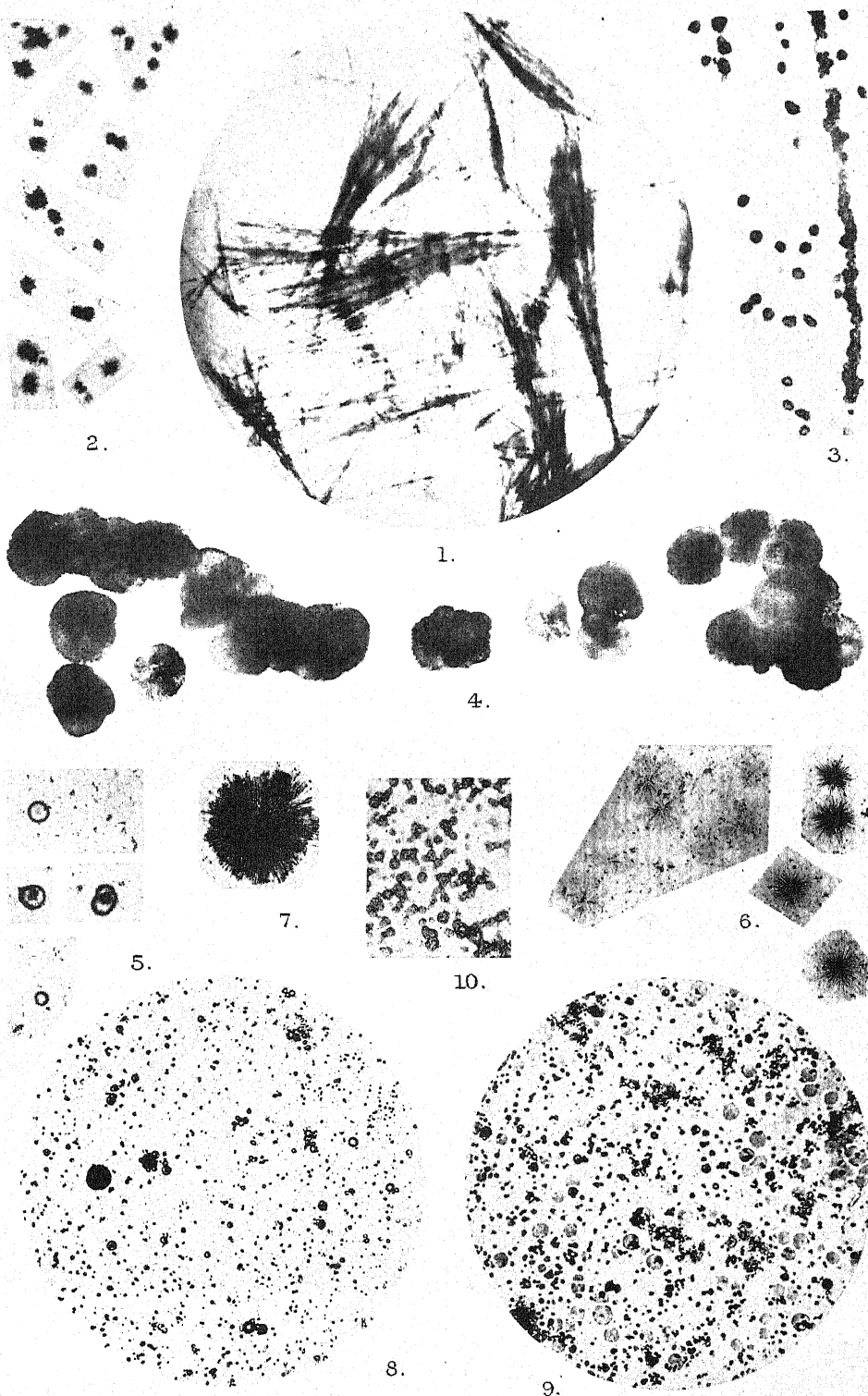
Fig. 7. An individual crystal cluster of maltose phenylosazone, showing blunt-ended crystals.  $\times$  *circa* 180.

Fig. 8. Drops of syrup yielded by 2 per cent. of maltose phenylosazone, after being heated in glycerine and allowed to cool. Photographed four months after heating.  $\times 500$ .

Fig. 9. From a 3 per cent. mixture of maltose phenylosazone in glycerine, heated and allowed to cool, showing drops of syrup and larger pale yellow spheres. Photographed four months after heating.  $\times 500$ .

Fig. 10. From a 1 per cent. mixture of maltose phenylosazone in glycerine after a second heating. Note the more crystalline appearance of the osazone. Photographed four months after heating.  $\times 114$ .





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# 'Brown Oak' and its Origin.

BY

PERCY GROOM, M.A., D.Sc.,

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THE ordinary heart-wood of certain individual trees of *Quercus Robur* in Great Britain is sometimes partially replaced by a firm, richer-toned, often reddish, brown wood known as 'brown oak' or 'red oak'. Such wood varies in tint from dull brown to rusty brown, or even to rust-coloured. Sometimes it is uniformly coloured, but at other times longitudinal bands of more or less normal-coloured heart-wood alternate with deep or bright brown streaks, which may include nearly black patches. This latter type of 'brown oak' is the so-called 'tortoiseshell' variety.

Trees possessing 'brown oak', so far as is known, show no external differences from normal individuals. This is not surprising as the heart-wood is the sole part affected, the remaining tissues being normal in structure and apparently in dimensions. In particular the sap-wood seems to be of ordinary size, and the sole evidence suggesting a possible precocious conversion of sap-wood into heart-wood is limited to the occasional presence of scanty starch-grains in a few cells of medullary rays in the heart-wood. Some 'brown-oak' trees are stag-headed. This condition induced by the desiccation of the branches is known to be caused by a number of influences that either check the supply of water to the foliage, or induce excessive transpiration. The evidence provided later in this paper proves that there is no reason to believe that the stag-headed condition of 'brown-oak' trees is directly induced by the influence responsible for the production of the brown heart-wood.

## GENERAL DISTRIBUTION OF 'BROWN' WOOD.<sup>1</sup>

In the trunk the brown wood most frequently occurs at the base, extends upwards to a variable height, and usually tapers in such a manner that its topmost portion apparently coincides with the inmost heart-wood. Not infrequently, however, in its upward taper the 'brown heart' becomes gradually confined to one side of the trunk. A special example of this was

<sup>1</sup> For many of the facts concerning the general distribution of 'brown oak' in the individual tree I am indebted to Messrs. Alexander Howard and Stuart Oliver, the former of whom informs me that he has seen 'brown oak' in a specimen of *Quercus rubra*.



afforded at Radlett by a tree, whose brown wood at the base of the trunk extended (apparently) completely across the heart-wood, then tapered very sharply in an upward direction, becoming at the same time confined to one side of the trunk, and continuing thus upwards, gradually tapering to extinction at an approximate height of 15 feet.

In connexion with this partially unilateral arrangement may be mentioned the interesting case of an oak-tree that grew near a stream. The bole, only 18 inches in height, gave way to two erect stems, each of which was about 18 inches in thickness over a length of 12-15 feet. The stumpy bole showed 'brown oak' on one side only, and the erect leader topping that side also was characterized by 'brown' wood, whereas the other leader springing from the half of the bole possessing normal heart-wood contained no 'brown oak'.

This case leads to the consideration of those pollard oaks in which the main trunk includes 'brown oak' extending up to the region of insertion of the branches, which form the multiple leaders of the crown. In the majority of cases 'brown oak' is contained by only one or, perhaps, two of the several leaders, and these are inserted above that section of the main trunk which is characterized by the strongest and richest colour in its brown heart; in such cases the remaining leaders show heart-wood normal in colour. Yet occasionally all the leaders (five in one case) include rich 'brown oak'.

'Brown oak' extending *upwards* in the trunk is usually arrested by extensive knots, and, in any case, a large knot acts as an obstacle. Consequently, in such cases, large boughs springing from the *side* of the trunk are apt to be devoid of 'brown oak'. The reason for this will be at least partly evident, when the cause of the browning of the heart-wood is explained later in this paper.

When 'brown oak' occurs at the base of the trunk it also extends downwards into the main root, where it tapers in the same manner as in the trunk.

Apparently 'brown oak' may occur in upper parts, including the crown, of a tree whose more basal parts are devoid of it. Very significant in relation to the cause is Mr. A. Howard's statement as follows: 'it appears probable that in a few cases the brown wood may start from a large knot below the crown, and extend somewhat downwards'. Mr. Stuart Oliver mentions one case in which the brown wood extended completely across the heart at the base of the bole, and upwards for some distance, then disappeared, only to recur at a height of 35 feet where the bole forked.

*Age of trees possessing 'brown oak'.* While on the one hand very old oak-trees contain 'brown oak', the minimum age at which the tree can acquire it is apparently determined by that at which the heart-wood

normally appears. Trees with a maximum diameter of trunk of 12 inches frequently contain such wood, which is to be seen in individuals whose maximum estimated age was twenty years.

'Brown-oak' trees in relation to site. Of oak-trees growing close together some may be normal, and others may be 'brown-oak' trees. For instance, at Farming Woods Park, of seven oak-trees close together, only one had 'brown oak'; whereas in a small wood at Stanmore in Middlesex the majority of oak-trees had thoroughly brown heart-wood, others had their heart-wood brown to a slight extent, and yet others were quite normal.

#### PREVIOUS HYPOTHESES AS TO THE CAUSE OF THE PRODUCTION OF 'BROWN OAK'.

Up to the present the cause of the production has been unknown, but three hypotheses have been put forward assuming that it is:

- (i) Due directly to some *chemical* body locally abundant in the soil.
- (ii) Due to *incipient decay*.
- (iii) A mere *sport*.

(1) The *chemical* hypothesis not only has no evidence in its favour, but is rendered improbable by the facts concerning the occurrence side by side on the same site of normal and 'brown-oak' trees, and concerning the distribution of 'brown oak' in the individual tree, and especially those cases of its unilateral or discontinuous distribution in the trunk. The chemical constituent frequently suggested is iron. Published analyses show that iron may be feeble or abnormally abundant in the wood, and yet this may be normal. An analysis conducted in the Chemical Laboratory of the Imperial College also shows that the amount of iron in 'brown oak' does not necessarily exceed that in normal heart-wood. The following was the result of the analysis:

|                                | Ash.   | Iron (as Fe). |
|--------------------------------|--------|---------------|
| Normal heart-wood . . . . .    | 0.17 % | 0.01 %        |
| Brown       ,,       . . . . . | 0.5 %  | 0.01 %        |

(2) *Incipient decay* has often been suggested as the cause of the appearance of 'brown oak'. Such a view seems to imply that the browning of the heart-wood is an early stage of a process which culminates in the rotting and disintegration of the affected wood. Calculated to arouse such a suspicion are the following facts: Freshly felled 'brown-oak' trees often show the brown heart in a condition of decay, or the trunk partly hollow. Occasionally, at the base of such trees, the heart-wood up to a height of from 3 to 6 feet is converted into a mass of wood rendered white by decay, while above this is firm and hard typical 'brown oak'. Finally, an interesting and significant fact is mentioned by Mr. Alexander Howard, namely

that often it is found that 'brown-oak' trees have lost their tap-root, whose functions are carried on by massive lateral roots.

Not one of these facts indicates definitely that the influence causing the rotting of the wood is identical with that causing the heart to become 'brown oak'. The facts merely indicate that in the case of these trees with rotten heart-wood, there are facilities for the entrance, through wounds, of fungi capable of attacking heart-wood of the oak, and that in certain cases those facilities are especially marked near the base of the trunk and roots. In the sequel it will be possible to discuss if the cause of browning and rotting of the wood is the same.

Deepening in the colour of wood, such as is undergone by alder, or by the heart-wood of larch, pine, and other trees, on exposure to air and light is assumed to be of a purely chemical nature, and at least often is due to oxidation (which is also largely responsible for the change of tint in sap-wood that is being converted into heart-wood). But, with the exception of the still unexplained cases of the 'red heart' of beech and of French oak (*Chêne rouge*), considerable deviations from the normal colour of the heart-wood are invariably due to the action of fungi. Yet no observer has recorded the presence of a fungus in firm 'brown oak' that shows no macroscopic symptoms of decay. And against the suggestion that a fungus or bacterium is responsible for the replacement of normal heart-wood by 'brown oak', is the fact that good 'brown oak' is to the timber-merchant or builder strong, hard, sound wood, responding to tools as does normal oak timber. It is true that species of *Ceratostomella*—for instance *C. Pini*—living in the sap-wood weakens the wood little or not at all, as its attack on wood-substance is slight; but in this case the fungus feeds on the carbohydrate and proteid food of the medullary rays and wood-parenchyma: such food is not generally available to any fungus living exclusively in heart-wood.

Therefore there was no adequate reason for believing that 'brown oak' owes its origin indirectly to a fungus or other foreign organism, or is the first step in a process of decay.

(3) The third hypothesis, namely that 'brown-oak' trees are mere sports, can be dealt with only after the evidence supplied in the sequel. Yet it may be pointed out that on such a hypothesis the occasional uneven distribution of 'brown oak' on the same site or in one and the same tree would find analogies in connexion with some tropical trees, including ebonies (e.g. *Diospyros Kurzii* and *D. Melanoxylon*), whose true dark heart-wood shows similar varied development.

#### TRUE CAUSE OF PRODUCTION OF 'BROWN OAK'.

As it seemed possible that the reason of our ignorance as to the cause of the production of 'brown oak' lay in the lack of any microscopical observations at the critical stage of its origin, I secured specimens of wood of freshly

felled 'brown-oak' trees. These came from different districts and were kindly supplied by Messrs. William Oliver & Sons, Messrs. Bradley, and Dr. Borthwick of Edinburgh. Two of the three specimens clearly showed wood in the process of conversion into 'brown oak'. Those specimens proved that 'brown oak' is confined to the heart-wood, and is not produced by a change in the sap-wood, but *first passes through the stage of being normal heart-wood*. The first visible macroscopic change is a superposition of a yellow coloration on the normal colour of the heart-wood; this is succeeded by a deepening of tint until the full-coloured brown stage is attained. With the naked eye in one specimen (Oliver's) it was possible to see that the browning took place in the heart-wood along longitudinal strands, causing the wood to be brown-striped in longitudinal section, and dotted with brown islands in transverse section.

**Proof of the presence of a living fungus in the heart-wood  
during conversion into 'brown oak'.**

Pieces of the freshly felled wood were placed under running tap-water for 6-12 hours, and then placed above water in sterilized potato-dishes. From the exposed transverse section pure white fungal hyphae grew forth, almost exclusively from regions of the heart-wood, where conversion into 'brown oak' was in progress. No hyphae came from the sap-wood. The Edinburgh specimen showed a solid core of 'brown oak', and practically no normal heart-wood; the hyphae thus emerged from the periphery of the 'brown oak' in close vicinity to the sap-wood. But Oliver's specimen showed in succession within the sap-wood a layer of normal heart-wood, one of brown-striped heart-wood, and a solid core of 'brown oak'. Accordingly the hyphae emerged solely from the periphery of the solid core, and also from the brown strips and peripheries of these in the striped wood.

The whiteness of the colour of the hyphae (apart from their distribution) indicated that they belonged to a fungus or fungi living in the wood, and did not result from accidental infection. To confirm this, other specimens, after treatment with running water, were externally sterilized by means of an alcoholic solution of corrosive sublimate, and were subsequently washed with sterilized water: yet the result was the same.

The white hyphae subsequently often became golden or tawny if they dried and remained sterile. Otherwise numerous green conidiophores soon arose, and resembled those of *Penicillium*. Later the conidiophores sometimes assumed a warm-bronze tint.

**Artificial production of 'brown oak'.**

In order to test the effect of the fungus on normal heart-wood, small cubical blocks of the latter were piled on top of one another in wide test-tubes, sterilized by steam, and infected by conidia of the fungus. The test-tubes were

kept upright, and in some cases the plugs of cotton-wool were covered with caps of tin-foil. At the bottom of the tube was previously sterilized water containing spores that had not adhered to any of the blocks. This arrangement ensured to the different blocks different degrees of moisture, the supply of moisture increasing from above downwards, the lowest block being permanently partly immersed in water.

The blocks in the middle of each column in the tube had the medium amount of moisture, and it was they that assumed a brown colour similar to that of 'brown oak' (in fact very similar to Messrs. Oliver's specimen), but varying towards that of fumed oak. The blocks partly immersed in water, and those at the top of the column in tubes, when no tin-foil caps were used, showed little or no change in tint. *The brown colour was assumed therefore only when the heart-wood contained moisture exceeding a certain minimum, and falling short of a certain maximum.* Such is one of the conditions of development in wood of all wood-destroying fungi.

In a second series of cultures in which larger boards of heart-wood were used, and the precautions against the intrusion of foreign organisms were less rigid, the boards showed the successive characteristic changes of tint from yellow to brown, including small patches of the rich brown of genuine 'brown oak'.

#### Distribution of the mycelium in the wood of the tree.

The colour of the hyphae emerging in culture from browning oak, the definite localization of these emerging hyphae, and the artificial production from heart-wood of wood simulating 'brown oak', all point to a causal connexion between fungus and browning process. This view is strengthened by the distribution and nature of the mycelium in the wood of the standing tree. Hyphae are absent from the sap-wood (and tissue outside it), and from parts of the normal heart-wood distant from the brown wood; they occur in an active living condition in regions of the heart-wood where conversion into brown wood is taking place, and, lastly, are present, mainly at least, in a dead and disguised form in 'brown oak' that has attained its final yet firm condition. The fact that in the mature brown oak the mycelium is wholly, or possibly nearly wholly, dead causes one to doubt if the decay observed in 'brown oak' is due to the same fungus; and additional reasons for this doubt will be given later in this paper.

#### STRUCTURE AND DEVELOPMENT OF 'BROWN OAK'.

##### (a) Sap-wood.

The structure and contents of the sap-wood are normal; starch was particularly abundant in the parenchyma, thyloses, and medullary rays.

In the sap-wood, normal and brown heart-wood, many fibro-tracheides

possessed a thick internal layer of the wall that assumed a faint lilac tint with iodine, a blue colour with iodized chloride of zinc, and refused to answer tests for lignified walls. The occurrence of this wall-layer in the sap-wood proves that in the 'brown oak' its lack of lignification is not due to fungal attack.

(b) **Normal heart-wood.**

The heart-wood near the sap-wood differed from this by the almost complete lack of starch (remnants of which occurred in isolated ray-cells and in thyloses of the large vessels), and by the abundance of tannin not only in all kinds of parenchyma, but also in the walls of the constituents, especially the fibro-tracheides, thyloses, and to a less extent ray-parenchyma.

(c) **Brown heart-wood.**

The 'brown oak' showed the same general distribution of tannin as in the normal heart-wood. But it also contained not only solid, including brown, substances in various constituents of the wood, but also hyphae.

For the purpose of cutting sections, the wood was softened by treatment with 50 per cent. commercial hydrofluoric acid (after being boiled and cooled repeatedly in water) and the wood blocks were eventually kept in a solution of glycerine, alcohol, and water. The sections were further treated with water if mounted in glycerine jelly, and, if mounted in Canada balsam, were for a time immersed in absolute alcohol and xylol.

After such drastic treatment normal heart-wood had almost or entirely lost its tannin, and showed no solid nor coloured contents. 'Brown oak', on the contrary, even in its incipient stage still showed tannin in its walls and in certain solid brown bodies that occurred in various structural constituents. These brown bodies agree in reactions with the so-called 'wood-gum' or 'wound-gum' of wood.

1. The substance is insoluble, and does not swell appreciably in water, alcohol, xylol, concentrated hydrochloric acid (12 hours), concentrated sulphuric acid (12 hours), equal parts of concentrated ammonia and caustic potash solution (12 hours), nor successively in the last named and strong hydrochloric acid. (In the case of brown oak that had reached its final condition and was treated with concentrated sulphuric acid, there seemed to emerge from the brown bodies in question bubble-like drops of a colourless substance. These drops were probably derived from fungal hyphae concealed within the brown substance.)

2. With phloroglucin and hydrochloric acid the brown substance sometimes assumed a carmine colour and thus agreed with typical wood-gum, but even in such cases it refused to respond to Mäule's test for lignification; in other cases it remained yellow with phloroglucin and hydrochloric acid. Possibly the substance is never lignified, and owes its occasional response to

the phloroglucin test to the presence of one of the number of substances capable of causing the carmine coloration.

3. The substance is singly refractive.

As the chemical nature of such wood-gum (in wound-wood, true heart-wood, and false heart-wood) is unknown, and as it is not wood and there is no evidence that it is gum, in the sequel the substance in question will be termed 'the brown substance'.

Tannin is present in the brown bodies under discussion. To its presence they owe their blue-black coloration in ferrous sulphate, and deep staining in methylene blue, lactic blue; also their deepened and lightened tint in caustic potash and hydrochloric acid respectively. But tannin is not an essential part of the substance in question, as parts of one and the same brown body are respectively devoid and possessed of tannin.

As the term 'tannin' is here used merely to indicate a substance that responds to the test for certain tannin-bodies by assuming (in this case) a blue or blue-black colour with ferrous sulphate, it follows that the tannin present in the brown heart-wood is either different from that of normal heart, or if identical is in larger quantity, or is held more firmly by the walls (and brown substance). The second possibility is excluded by the analysis given later in this paper.

#### BIOLOGY AND EFFECTS OF THE FUNGUS.

For the sake of clearness, the succeeding description refers to wood that had been subjected to the treatment (boiling, hydrofluoric acid, and so forth) which has been described, and which had removed the tannin more or less completely from cells not changing nor changed into 'brown oak'.

##### (a) Heart-wood of normal colour.

Immediately within the normal heart-wood (devoid of hyphae) was a region of the same colour and for the most part free from hyphae, yet here and there showing some of these in vessels and wood-parenchyma. These hyphae were often dotted with glistening globules, but no masses of 'brown substance' occurred. Nearer to the more central 'brown oak' the main mass of the wood was devoid of hyphae and brown substance, though both of these showed (in tangential sections) sporadic patches of cells in the medullary rays containing hyphae and the 'brown substance', *which was yellow*.

Still nearer to the 'brown oak' the heart-wood, either normal or more yellow in colour, showed hyphae and brown substance arranged in longitudinal strands, and in some medullary rays, while the remaining tissue was normal in contents. This distribution of hyphae and brown substance in longitudinal strands thus is preparatory to the later stage already described, in which the wood is traversed by brown stripes. Both are due to the fact

that the hyphae advance most rapidly in a longitudinal direction in the vessels and circumvasal tissue, which form radial series. In a transverse plane advance of the hyphae is most rapid in a radial direction by means of the medullary rays.

These facts, including the slow advance of the hyphae in a tangential direction, help to explain the cases where the 'brown oak' advances and tapers from the base of the trunk upwards, or where it becomes restricted to one side or one of two stems in a double-stemmed oak, though in the first case the taper of the heart-wood itself may intervene. Further, the mode of advance of the fungus (coupled with the very feeble power that the fungus has of attacking lignified walls) at least partially accounts for the obstructive influence of large knots.

(b) **Brown heart-wood mainly in the form of longitudinal strands.**

In this stage and the preceding one in which the hyphae and brown substance are in strands, though not clearly marked to the naked eye, all steps in the advance of the hyphae and in the manufacture of the brown substance are to be seen. Both stages will therefore be described together.

The *advance of the hyphae along the medullary rays* was revealed especially clearly in the normal-coloured heart-wood in places where hyphae and brown substance were abundant in the ray, but absent or scanty in the neighbouring vessels, tracheides and parenchyma. In the rays the hyphae run mainly in a transverse radial direction, passing through the copious pits of the terminal cell-walls. Yet here and there they emit branches to the tissue on their flanks; for often the wood-parenchyma in the immediate vicinity of infected ray cells also contained hyphae and brown substance, whereas wood-parenchyma tangentially more remote lacked these.

In uniseriate and multiseriate rays alike the cells containing the brown substance always entertained hyphae, which could be traced farther out in the ray towards the normal wood than could the brown substance. With this exception very few of the colourless ray-cells in the infected region contained hyphae. These facts show that *the brown substance is the effect, not the cause, of the presence of hyphae*. In the medullary rays of the Edinburgh specimen the hyphae extended outwards approximately to the same distance as the first granular traces of the brown substance.

*In the vessels, tracheides, and wood-parenchyma the course of the hyphae is generally longitudinal.* Consequently in transverse section there are isolated islands of vessels and surrounding tissue containing hyphae and brown substance. The actually contiguous uniseriate rays may be devoid of hyphae or contain these only where the ray actually crosses the infected island. Traced further outward such a ray is devoid of hyphae until another infected island is touched, when hyphae may reappear in it. Thus as they travel along the vessels and circumvasal tissue hyphae can infect



rays at successive levels. Such a mode of infection was particularly clear in the case of multiseriate rays, which crossed infected strands of vessels and showed hyphae and brown substance merely in their sides towards the infected vessels.

Occasional hyphae were seen running tangentially in tracheides to or from other tracheides and a medullary ray.

### (c) Production of the brown substance.

The following stages were observed in the hydrofluoric-acid material:

1. Hyphae entering colourless parenchyma cells or vessels were studded with glistening globules. And in the Edinburgh material such was the case with parts of the hyphae freely traversing the lumen of the cell, and not in contact with the cell-wall.

2. Cells showed granular colourless, or very faintly yellow, contents in proximity to the active intracellular young hyphae.

3. The contents were definitely yellow, in larger quantity, and formed a homogeneous hyaline mass surrounding the hyphae.

4. In the final stage, as seen in completely 'brown oak', the substance is definitely brown, present in still greater quantity, and may fill the parenchyma-cell, except where it is permeated by dead, often almost unrecognizable, hyphae, which are separated from it by a clear space.

These stages indicate that the brown substance is deposited outside the hyphae as colourless globules, which later increase in number and undergo some change so as to give rise to a yellow mass of granules or globules; these in turn remain in or assume a colloid condition to form eventually a homogeneous solid mass of constantly deepening colour. The appearances presented suggest that the substance is excreted by the fungus, but an alternative suggestion is given in the sequel where the food of the fungus is discussed.

### (d) An additional plugging substance.

Mention must be made of an entirely different solid substance of unknown nature present even in the material, subject to treatment with hydrofluoric acid and so forth. This substance, granular in nature, when seen in thin sections often showed a yellowish tinge, but when seen in thicker masses was dark and opaque. Its occurrence and distribution were independent of those of the hyphae. It occurred in normal heart-wood as abundantly as in brown heart-wood. In distribution it was localized, often being found in longitudinal strips, especially in wood-parenchyma and ordinary tracheides, but also in the adjoining thick-walled fibro-tracheides. In such cases it was present in the uniseriate rays traversing the strip solely where the former crossed the latter.

(e) Action of the fungus on lignified walls.

The effect of the fungus on the mechanical strength of the heart-wood is so small that 'brown oak' is an excellent material for panelling and furniture. This corresponds to the fact that the fungus attacks lignified walls as visible structures feebly and slowly. It appears to pass from one cell or vessel to another solely through the pits. This was particularly evident in the case of hyphae traversing the terminal walls of wood-parenchyma and ray-parenchyma; and cases were observed in which a hypha on reaching a spot on the wall where no pit occurred executed a bend and so reached the nearest pit.

The constituents of the wood for the most part retain not merely their visible structural integrity but also their lignified condition. Yet the fungus has some power of delignifying wood. Here and there where two vessels, or a vessel and a tracheide, or two tracheides, were in lateral contact, the half of the wall belonging to the constituent containing hyphae gave a cellulose reaction in the vicinity of these, but a lignified reaction on the side towards the constituent devoid of fungi. In a more advanced stage of attack the wall was locally delignified throughout its thickness. This restricted power of delignification appears to begin at the pits, for the section of a wall separating two tracheae sometimes showed alternate minute patches of cellulose and lignified substance, each patch embracing the whole thickness of the wall. Each cellulose patch probably corresponded to a pit whose plane lay outside the section. Occasionally thin sections showed real gaps in the walls separating two vessels, although I never succeeded in proving beyond doubt that these were due to the fungus, and not to the razor. Probably owing to its weak power of attacking lignified walls, and its exclusive or nearly exclusive passage through pits, the fungus occurs very scantily in fibro-tracheides.

(f) Source of nutriment of the fungus.

As the fungus is confined to the heart-wood, and makes so slight an attack on the visible structure of the lignified walls, the question arises as to the source of its food. Available are: in the lignified walls, pentosans (xylan and so forth), pectic bodies, glucosides, and tannin, as well as the cellulose and substances causing the 'lignin' reactions. Tannin is also available in lumina of the parenchyma, including that of medullary rays, and thyloses. Whatever be the precise food substances utilized, the *method of nutrition of this fungus is novel*, so far as our present knowledge is concerned, though the future will probably reveal other wood-inhabiting fungi of similar feeding habits, possibly associated with the inception of firm wound-wood or firm false heart-wood.<sup>1</sup>

<sup>1</sup> See E. Münch, 'Über krankhafte Kernbildung'. Naturwiss. Zeitsch. f. Forst- und Landwirtschaft, 1910, Heft 11.

Several sets of facts favour the view that *tannin* is used as food-material.

1. An analysis of 'brown oak' compared with the normal heart-wood showed that the latter contained 13.33 and the former 10.05 per cent. of tannin. This would represent a loss of nearly 25 per cent. of the original tannin. But in the absence of analyses of oak timber at different depths inwards in ordinary heart-wood, it is conceivable that the smaller amount in the brown oak is not due to the fungus nor associated with the 'browning'.

2. The distribution of the fungus and tannin are of significance in this connexion. (For purposes of observation the wood was softened by dilute glycerine, induced to enter by means of an exhausting air-pump: no heat was applied.)

In the fully brown heart-wood tannin was generally diffused in the lignified walls, but especially in the lumina of the wood-parenchyma, thyloses, and cells of the medullary rays. In all uniseriate and multiseriate rays there was tannin, which often was most abundant in the marginal cells and lacking from many other cells. It was in all three forms of parenchyma that the hyphae and brown substance were also most abundant. In one and the same cell frequently parts of the brown substance contained tannin, which was absent from other parts of the same mass in the same cell.

In regions where conversion of the heart-wood into 'brown-oak' was taking place some of the cells of the wood-parenchyma and medullary rays were poor in tannin, or devoid of it, and at the same time free from hyphae; whereas contiguous cells in the same tangential or radial series contained hyphae and richer stores of tannin. Again, in some cells there was no tannin except a thin film in (or on?) the hyphal wall, or isolated minute droplets studding the hypha, which sometimes also traversed a larger globule of tannin: in these cases the tannin stained unusually light blue (a somewhat dark cobalt-blue) with ferrous sulphate, yet was sufficiently concentrated to stain deeply with lactic blue.

These facts are all capable of two opposed interpretations, namely that the hyphae consume or excrete tannin. The view more consistent with evidence derived from other sources is that the hyphae preferentially enter tannin-containing cells, and consume the tannin until in the absence of fresh supplies tannin is so reduced in quantity as to be a dilute solution in the form of a film or minute globules on the hypha. After that stage the tannin may be wholly absorbed. But in addition to the tannin present in the cells, there is that in the walls; this may be liberated from the wall by the solution of some ingredients in the wall or by water or a solution excreted by the fungus, and may be deposited in the accumulating brown substance.

This view that the fungus feeds at the expense of the tannin is strengthened by *cultures* made of the fungus *in solutions of tannin*. Conidia of the fungus were sown in 0.05, 0.25, 0.5 per cent. solutions of commercial

tannin (which is not identical with the tannin of oak wood). When bacteria were excluded vigorous submerged mycelia developed, but the solutions did not darken in tint. When the mycelia were accompanied by bacteria, derived from cultures from the original wood, the solutions darkened. Inside 'brown oak' I failed to find any bacteria.

#### IDENTITY OF THE FUNGUS.

Repeated trials as to the source of the *Penicillium*-like conidiophores emerging from incipient 'brown oak' showed that these belonged to the fungus causing the process of browning. This was confirmed not only by the physiological action of the mycelia in cultures made in oak heart-wood, but also by the occurrence of minute *Penicillium*-like conidiophores in the narrow vessels of the intact 'brown oak'.

As regards other stages of the life-history of the fungus, I did not succeed in obtaining by means of cultures derived from conidial infections any other stage. But from the regions where the hyphae were still active on samples of the 'brown oak' from England and Scotland there were produced little spheroidal brownish-yellow fructifications, whose diameter did not exceed that of half a pea-seed. These appeared to belong to the Plectobasidiinae. Being unable to identify these, I submitted them to Mr. George Massee, who states that they are the basidiocarps of *Melanogaster variegatus*, Tul., var. *broomianus*, Berk.

#### SUMMARY.

In certain individual British oak-trees (*Quercus Robur*<sup>1</sup>) the ordinary heart-wood is partially replaced by a rich-toned, often reddish, brown wood, which is firm and hard, and is termed 'brown oak'.

Under the influence of a septate fungus living exclusively in the heart-wood normal heart-wood is changed in 'brown oak'. The fungus therefore presumably infects solely, through a wound, trees sufficiently old to possess heart-wood.

'Brown oak' usually occurs at the base of the trunk and the adjoining root, and generally tapers upwards in the stem and downwards in the root. But the fungus can gain entrance to upper parts of the tree and so produce in these regions masses of 'brown oak', even in individuals devoid of it in their lower parts.

The fungus in the infected tissue is responsible for the production of a brown substance (or brown substances) highly resistant to solvents and responding to the reactions of the ill-defined material termed 'wound-gum' or 'wood-gum'. This arises in the form of colourless or faintly yellow globules or granules, which eventually aggregate to form brown masses in

<sup>1</sup> This name is used in its main historic sense as including *Q. pedunculata* and *Q. sessiliflora*. From which of these species my material was derived, and the extent to which 'brown oak' occurs in the two, are unknown to me.

the cavities of the wood-constituents. The change of tint of the heart-wood as a whole and the production of the brown substance in the individual cells definitely succeed the entry of the hyphae. Artificial infections of fragments of normal heart-wood caused this to assume colours approximating to or agreeing with those of true 'brown oak'.

The fungus (and colour change) advances most rapidly in a longitudinal direction along the lines of vessels and circumvasal tissue, and in a transverse direction along the medullary rays: the advance in a tangential direction is comparatively slow. These facts find at least partial explanation, first, in the extremely limited power of the fungus to attack and delignify lignified walls, and in the consequent advance from constituent to constituent exclusively through pits or pores; secondly, in the circumstance that the fungus thrives particularly in parenchyma (wood and ray) in which it runs mainly in the direction of the long axes of the cells, passing out through the numerous pits in the terminal walls.

Among the consequences of the mode of advance and limited power of dealing with lignified walls are the following:

(a) 'Brown oak' can remain firm and hard in the tree for a long time. And since the mycelium of the fungus concerned in mature 'brown oak' is largely, if not entirely, dead (even though conidia may occur in it) there is no reason to believe that the obvious decay of 'brown oak' occurring in some cases is due to this fungus. Such decay may be induced by other wood-destroying fungi that attack normal heart-wood of the oak.

(b) In early stages of conversion of heart-wood into 'brown oak' the latter is seen in its incipient condition as longitudinal darker bands traversing normal coloured wood. This condition is reflected in and explains the tortoiseshell variety of mature 'brown oak'.

(c) The advance of the process of browning is arrested or obstructed by large knots, though burr-wood with numerous small knots may be completely brown.

(d) Associated, at least partly, with the limited power possessed by the hyphae of advancing in a transverse and above all in a tangential direction, are the cases where brown oak becomes limited to one side of a stem, or to one or two among several 'leaders' into which the infected trunk divides.

The source of the food of the fungus constitutes a complex chemical problem at present insoluble by microchemical methods. That tannin is one of the sources is suggested, first, by the development of the fungus particularly in tannin-containing constituents of the heart-wood; secondly, by the power of the fungus to obtain all its essential organic food from commercial tannin; and, thirdly, by the smaller quantity of tannin in 'brown oak' than in the adjoining normal heart-wood (of the one specimen investigated).

The fungus responsible has conidiophores closely resembling those of

*Penicillium*. On incipient 'brown oak' of Oliver's and Borthwick's specimens there eventually were produced small spheroidal basidiocarps which Mr. George Massee identifies as *Melanogaster variegatus* var. *broomianus*. The identity of the conidiate fungus with that of the basidiate one was not established by pure cultures. If, however, the wholly or partially subterranean species of *Melanogaster* in question is the fungus responsible, infection by hyphae or spores through a wound in or near the root would appear to be especially simple, and might be partly responsible for the preponderance of 'brown oak' near the base of the tree. In this connexion may be mentioned the fact that the main root of 'brown-oak' trees is often found to be destroyed.

That the production of 'brown oak' is not due to the direct action of a particular chemical ingredient of the soil is proved by the distribution of this wood in the individual tree as well as by the occurrence side by side of normal and 'brown-oak' trees.

In conclusion I express my thanks to Professors H. Brereton Baker, F.R.S., and J. F. Thorpe, F.R.S., for securing quantitative estimations of the iron and tannin respectively, and to Mr. W. P. Rial for performing the analysis in the case of the latter; to Messrs. Alexander Howard and Stuart Oliver for valuable information; to Messrs. Borthwick, Bradley, and E. T. and S. Oliver for kindly supplying fresh specimens; and to Mr. George Massee for his identification of the fungus.

## APPENDIX.

BY MR. W. P. RIAL.

### Tannin in Oak heart-wood.

The wood was taken in the form of fine shavings across the grain: 9 grammes of this were placed in an extracting apparatus and extracted for 1 hour with 225 c.c. water. The residue was then extracted for another hour with 225 c.c. water, and at the end of this time a few drops of the liquid which was passing through the wood were tested with  $\text{FeCl}_3$  and found to contain no tannin. The extract was placed in a 500 c.c. measuring flask and made up to 500 c.c.

This was done for both specimens in a similar manner.

The tannin present was estimated by the method used at the Yorkshire College.

25 c.c. of an indigo carmine solution are added to 750 c.c.  $\text{H}_2\text{O}$  and  $\text{KMnO}_4$  added until a pure yellow colour is obtained.

The titration is then carried out in presence of 5 c.c. of tannin extract, and also in presence of 20 c.c. of pure tannin solution.

## RESULTS.

|                                  |  |
|----------------------------------|--|
| 25 c.c. indigo solution required | $\left. \begin{array}{l} 43.9 \\ 44.1 \end{array} \right\} 44.0 \text{ c.c. KMnO}_4$ |
| „ + 5 c.c. extract ordinary oak  | 67.5 c.c. „  |
| „ + „ „ brown                    | 61.7 „ „   |
| „ + 20 c.c. pure tannin solution | 61.5 „ „   |

The pure tannin solution was made by dissolving 0.1118 gm. of pure tannin in water and making up to 250 c.c.

$$20 \text{ c.c. pure tannin solution} \rightarrow 61.5 - 44.0 = 17.5 \text{ c.c. KMnO}_4$$

$$\text{i.e. 1 c.c. KMnO}_4 \rightarrow \frac{20}{17.5} \times \frac{0.1118}{250} \text{ gm. tannin.}$$

$$= 0.00051 \text{ gm. tannin.}$$

$$5 \text{ c.c. ordinary oak extract} \rightarrow 67.5 - 44 = 23.5 \text{ c.c. KMnO}_4$$

$$23.5 \text{ c.c. KMnO}_4 \rightarrow 23.5 \times 0.00051 \text{ gm. tannin.}$$

$$\therefore 500 \text{ c.c. „ „}$$

$$23.5 \times 100 \times 0.00051 \text{ gm. tannin,}$$

and this is contained in 9 gm. of wood.

$$\therefore \% \text{ tannin} = \frac{23.5 \times 100 \times 0.00051}{9} \times 100$$

$$= 13.33.$$

$$5 \text{ c.c. brown oak extract} \rightarrow 61.7 - 44 = 17.7 \text{ c.c. KMnO}_4$$

$$17.7 \text{ c.c. KMnO}_4 \rightarrow 17.7 \times 0.00051 \text{ gm. tannin.}$$

$\therefore 500 \text{ c.c. contain } 100 \times 17.7 \times 0.00051 \text{ gm. tannin, and this is contained in 9 gm. wood.}$

$$\therefore \% \text{ tannin} = \frac{17.7 \times 100 \times 0.00051}{9} \times 100$$

$$= 10.05.$$

# On the Structure and Development of the Secretory Tissues of the Marattiaceae.

BY

CYRIL WEST, B.Sc., F.L.S.

With Plate XVIII and fourteen Figures in the Text.

THE Marattiaceae are characterized by the possession of secretory tissues of two kinds, which have been called mucilage-canals, and also cells or ducts containing tannin. Owing to their prominence in this group of Ferns these tissues have received the attention of many botanists, the first reference to them appearing as early as 1847, when Karsten (11) observed mucilage-canals in the stem, leaves, and roots of the Marattiaceae and suggested that the cells bordering on these canals took part in the secretion of the mucilage.

Harting (7) distinguished between the ramifying canals with a definite epithelium of small cells and the simple intercellular canals which he observed in a doubtful species of *Angiopteris*.

Two kinds of mucilage-canal were also described and figured by Frank (10), who contrasts those which occur in the outer thick-walled tissue of the petiole of *Angiopteris* with those which are found in the inner thin-walled tissue. To the former he ascribes a lysigenous origin, but to the latter, which are wide-lumened and are lined with an epithelium of small iso-diametric cells, he attributes a schizogenous origin.

As a result of his investigations on species of *Angiopteris* and of *Marattia*, Trécul (21) arrived at a very similar conclusion, but maintains that the secretory elements in the fibrous zone of the petiole consist of a series of superposed large elongated cells containing tannin, while the true mucilage-ducts, although they arise schizogenously, possess only a transitory epithelium.

Van Tieghem (20) found both mucilage-ducts and tannin-sacs in the root and petiole of *Marattia laevis*. According to this author the former (mucilage-ducts) are lined with an indefinite epithelium of small irregular cells, from which the mucilage is derived. He adds, however, that here and there the ordinary large cells of the cortex abut directly upon the canal. In the root of *Angiopteris evecta* no mucilage-canals were found.



Russow (18) accepted Frank's explanation of the development of two kinds of canal, which arise by a schizogenous and a lysigenous process respectively.

In the course of his investigations on the anatomy of the Marattiaceae, Kühn (13) gave an account of the lysigenous development of the mucilage-canals in the roots of *Angiopteris*, *Marattia*, and *Kaulfussia*.

But the first comparative account of the development of the mucilage-canals of the Marattiaceae was published by Brebner (2), who arrived at the conclusion that they are schizogenous intercellular spaces, which develop much in the same way as the well-known resin-canals of *Pinus*, *Hedera*, &c. A well-defined living secretory epithelium is generally developed around these intercellular spaces. In a more recent communication (3) this author still maintains this view.

Lutz (14) published a full account of the development of the mucilage-canals in *Angiopteris evecta* and in *Marattia fraxinea*, var., and attempted to reconcile the conflicting statements of the earlier investigators. This botanist distinguished between the typical mucilage-canals, which develop schizogenously, and a second type of mucilage-canal, which arises by the solution of the terminal parting-walls of rows of tannin-cells. The tanniniferous contents of the latter are said to be gradually replaced by true mucilage.<sup>1</sup>

Farmer and Hill (9), on the other hand, ascribed a lysigenous development to the mucilage-canals which occur in the young sporophyte of *Angiopteris evecta*.

In his well-known memoir on the Psaronieae and Marattiaceae Rudolph (17) alludes to and figures (l.c., p. 196, Taf. iii, Fig. 2 a) both types of secretory tissue described by Lutz.

Campbell (4) observed lysigenous mucilage-canals in the stem of *Danaea elliptica* and of *Danaea Fenmani*, but states (l.c., p. 181) with reference to *Kaulfussia* that the lysigenous origin of the canals is less evident than in *Danaea*, while it is not impossible that they may sometimes be of schizogenous origin.

Charles (6) concluded that the mucilage-ducts in *Marattia alata* originate both schizogenously and lysigenously, generally the former.

In view of the striking lack of agreement in existing accounts of the structure and development of the secretory tissues of the Marattiaceae, which suggests variation or vagueness in these structures, a reinvestigation of this point seemed desirable, since it might throw some light on the much-debated subject of the phylogeny and affinities of this group of Ferns (cf. Matte, 15, p. 206; Seward, 19, p. 217), including the interrelationships of the constituent genera.

With this end in view the present investigation was undertaken.

<sup>1</sup> The results obtained by Lutz were adopted by Bitter (1) in his account of the Marattiaceae in Engler and Prantl, *Die natürlichen Pflanzenfamilien*.

## MATERIAL AND METHODS.

Material of the following genera and species was examined :

|   |                                      |
|---|--------------------------------------|
| <i>Angiopteris evecta</i> , Hoffm.            | <i>Kaulfussia aesculifolia</i> , Bl. |
| <i>Archangiopteris Henryi</i> , Chr. et Gies. | <i>Marattia alata</i> , Sw.          |
| <i>Danaea alata</i> , Sm.                     | <i>Marattia attenuata</i> , Lab.     |
| <i>Danaea nodosa</i> , Sm.                    | <i>Marattia Cooperi</i> , Mre.       |
| <i>Danaea simplicifolia</i> , Rudge.          | <i>Marattia fraxinea</i> , Sm.       |

In order to eliminate at least one possible source of error, part of this material was carefully fixed in chromo-acetic or in acetic alcohol (glacial acetic acid, 1 part : absolute alcohol, 3 parts). The remainder was fixed in 70 per cent. alcohol.

For this investigation serial sections were necessary, therefore microtomed sections were cut ( $6\mu$ – $12\mu$ ), but care was taken to check the work by examining thick hand-sections.

Various stains and reagents were employed, including safranin, gentian-violet, eosin, methylene blue, iodine, ferric chloride, Congo red, and haematoxylin.<sup>1</sup> These were used singly and in combination.

### 1. *Development and Structure of the Mucilage-canals.*<sup>2</sup>

#### Method i. Protogenetic Lysigenous<sup>3</sup> Mucilage-canals.

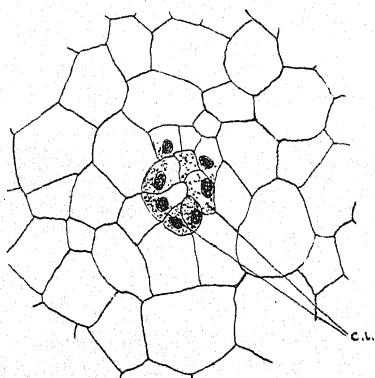
The mucilage-canals of the Marattiaceae usually arise on the first differentiation of tissues at the growing-point of the stem or root. In the leaf also the very early differentiation of the canal-initials was observed.

Both transverse and longitudinal sections through the young tissues of the stem, root, or leaf of by far the largest number of genera and species examined reveal scattered groups of cells, the canal-initials, which remain meristematic after the neighbouring cells of the ground-tissue have passed over into the permanent condition. These specialized cells usually divide into 2, 4, or 6 without any appreciable increase in size ; as a result of this cell-division groups of cells, which can readily be distinguished from the surrounding cells not only by their smaller size but also by their relatively larger nuclei and denser cytoplasm, are produced (Text-figs. 1–4 ; Plate XVIII, Figs. 6 and 12).

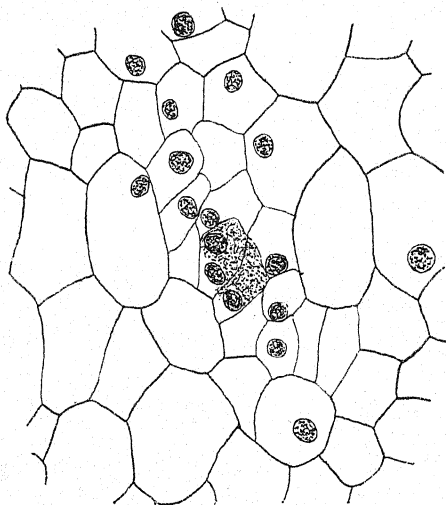
<sup>1</sup> This reagent was made up according to the formula published by Kleinenberg in Quart. Journ. Micr. Sci., lxxiv, 1879, p. 208.

<sup>2</sup> In this paper the term 'mucilage' is applied to a substance (or substances) of unknown chemical composition, which exhibits certain recognized physical properties. For an account of the optical properties of various plant mucilages, including that of *Angiopteris*, see Schwendener, S., 'Nochmals über die optisch-anomale Reaction des Traganth- und Kirschgummis,' in Sitzungsber. d. Akad. d. Wiss. zu Berlin, Bd. ii, 1890, p. 1131.

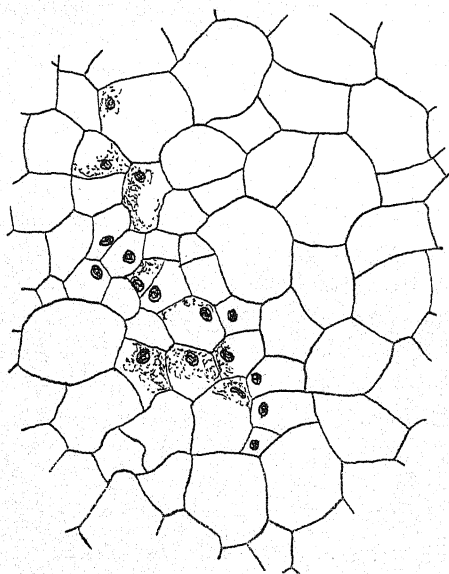
<sup>3</sup> Frank, l. c.



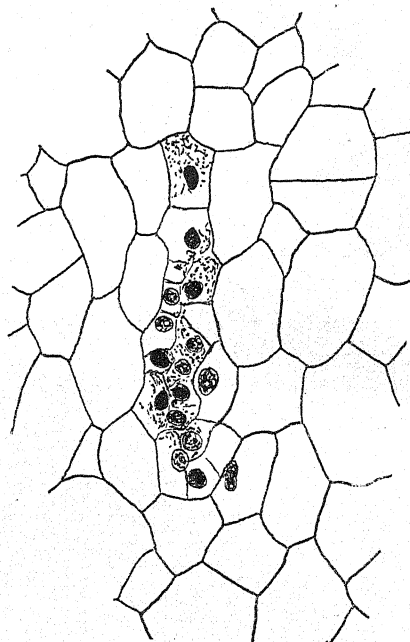
TEXT-FIG. 1. Early stage in development of mucilage-canal in the stem of *Angiopteris evecta*, Hoffm.  $\times 350$ . c.i. = canal initials. The shaded areas represent protoplasm in process of mucilaginous degeneration.



TEXT-FIG. 2. Early stage in development of mucilage-canal in the petiole of *Danaea nodosa*, Sm.  $\times 380$ . The shaded areas represent protoplasm in process of mucilaginous degeneration.



TEXT-FIG. 3. Early stage in development of mucilage-canal in the stem of *Marattia fraxinea*, Sm.  $\times 350$ . The shaded areas represent protoplasm in process of mucilaginous degeneration.

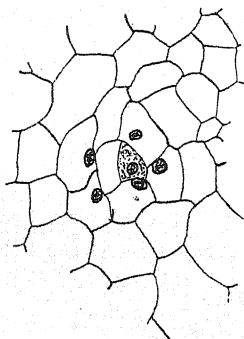


TEXT-FIG. 4. Early stage in development of mucilage-canal in the stem of *Kaulfussia aesculifolia*, Bl.  $\times 350$ . The shaded areas represent protoplasm in process of mucilaginous degeneration.

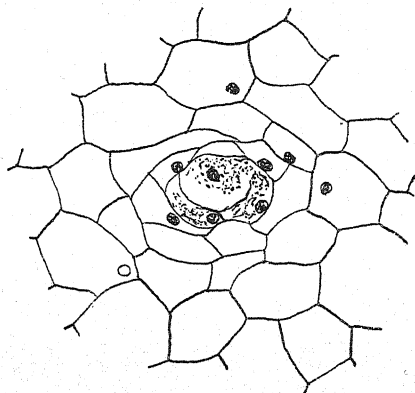
The arrangement of the small cells constituting such a group is usually very irregular (Text-figs. 2-4; Figs. 6 and 12).

Most botanists have agreed as to the earlier stages in the development of the mucilage-canals, but much confusion has arisen in the past as a result of the difficulty of correctly interpreting the subsequent stages.

Whereas some (e.g. Frank, Brebner, Lutz) have maintained that the cavity of the duct originates by the splitting apart of the walls of these small cells, which persist as a definite epithelium lining the intercellular space into which they actively secrete mucilage, others (e.g. Kühn, Farmer and Hill, Campbell) have ascribed a lysigenous origin to the canals and have asserted that the cavity is formed by the collapse and disorganization of these groups of cells, which become converted into mucilage.



TEXT-FIG. 5.



TEXT-FIG. 6.

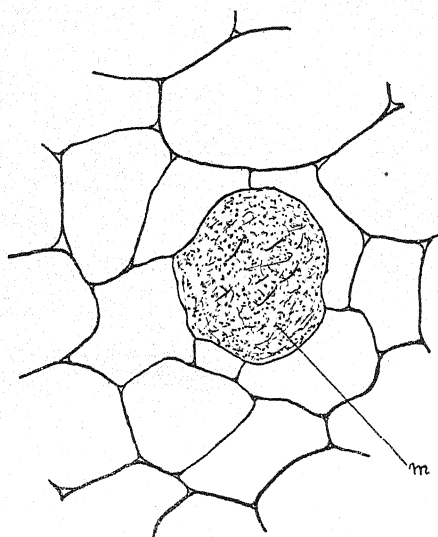
Early stages in development of mucilage-canals in the stem of *Danaea alata*, Sm.  $\times 350$ .  
The shaded areas represent protoplasm in process of mucilaginous degeneration.

My own observations, which extend over five genera and many species, for the most part confirm the explanation given by the latter group of botanists.

The cytoplasm of certain cells of the group undergoes local mucilaginous degeneration (Text-figs. 1-5; Figs. 6 and 11-14). Kleinenberg's haematoxylin was found most satisfactory for demonstrating the changes which take place in the cytoplasm, since it clearly differentiates between the unaltered cytoplasm and that which is undergoing mucilaginous degeneration. This process usually begins near the common point of contact of the cells, and gradually extends through the cytoplasm until finally the entire contents of the cell, including the nucleus, are involved in the change. The cell-contents meanwhile undergo a marked contraction and frequently come away from the cell-wall (Figs. 6 and 13).

In this way a condition is attained which might easily be mistaken for a splitting apart of the cell-wall, especially when it is remembered that

at this stage the cell-wall is also undergoing mucilaginous degeneration and can be seen only with difficulty in thin microtomed sections. However, the cell-wall can still be identified as a very thin line extending across the clear space produced by the contraction of the cytoplasm. It is worthy of note that Brebner (l. c., p. 447) observes that 'in the young developing canals of the frond of *Angiopteris evecta*, *Marattia alata*, and *Marattia cicutaefolia*, there is little or no sign of mucilage, for there does not seem to be anything more highly refractive than the mounting medium in the schizogenous space, nor anything which stains appreciably with safranin or haematoxylin, whereas in the adult condition the cavity is filled with a substance, "mucilage", which stains strongly with these reagents'.



TEXT-FIG. 7. Transverse section of an adult mucilage-canal from the petiole of *Archangiopteris Henryi*, Chr. et Gies. Note complete absence of epithelial cells.  $\times 380$ . *m.* = mucilage.

Charles (l. c., p. 96) also states that 'the space left when the walls break apart appears empty at first, then becomes filled with a vacuolate substance that stains with aniline blue'.

Charles (l. c., p. 96) also states that 'the space left when the walls break apart appears empty at first, then becomes filled with a vacuolate substance that stains with aniline blue'.

The mucilaginous degeneration of the cell-wall proceeds apace until the cell finally collapses owing to the complete solution of the cell-wall.

In this way, then, the cavity of the duct originates (Text-figs. 4 and 6; Figs. 6, 8, and 12-14).

The rate at which individual cells become disorganized is very

unequal; thus, several cells which appear quite healthy may be observed bordering on the duct at a comparatively early stage in its development (Text-fig. 6; Fig. 13, and cf. the 'bridge-cells' of Brebner, 2, p. 446).

Sooner or later, however, they all share the same fate, their disorganized remains contributing to the mucilaginous contents of the duct, which is now surrounded by the unmodified cells of the ground tissue (Text-fig. 7; Figs. 7 and 9, *m.c.*).

Treatment with iodine, as recommended by Brebner (2, p. 447), failed to reveal the presence of an epithelial layer.

The important factor of tissue-tensions, which has been admirably elucidated by G. Kraus (12) and Newcombe (16), appears to have been completely neglected by all previous workers on the development of mucilage-ducts.

As mentioned above, these elements generally arise just behind the generative tissues of the stem and root and are consequently subjected to a variety of stresses. The stretching of cells due to turgor increases as they pass from the embryonal condition and decreases as they assume their permanent condition, and since the mucilage-ducts in the stem or roots are generally surrounded by parenchymatous cells with thin walls of cellulose, it follows that the latter will tend to contract when the force of turgor is withdrawn. The negative tension thus called forth is considerable, and may account for the very rapid increase in the diameter of the mucilage-ducts which occur in the stem and roots of most of the genera and species examined (Figs. 8 and 13).

The adult mucilage-ducts, which frequently branch and anastomose, pursue a very irregular course through the ground-tissue.

The above description applies to the development of the mucilage-ducts in the following genera and species :

*Angiopteris evecta*, Hoffm. (root excepted) (see also Methods ii and iii).

*Archangiopteris Henryi*, Christ et Gies. (the material of this plant was unsuitable for determining this point with accuracy).

*Danaea alata*, Sm.

*Danaea nodosa*, Sm.

*Kaulfussia aesculifolia*, Bl. (see also Method iii).

*Marattia alata*, Sw.

*Marattia attenuata*, Lab.

*Marattia Cooperi*, Mre.

*Marattia fraxinea*, Sm.

A slight variation from the usual type of development was sometimes observed in the roots of *Marattia Cooperi*. Here the mucilage-canals may originate from a single row of superposed cells with dense granular cytoplasm. These initial cells do not divide, but rapidly undergo complete mucilaginous degeneration. The nuclei of these cells appear to be remarkably resistant to the enzymes which cause the breaking down of the cells and their contents, and often retain their individuality for a very considerable period after the complete collapse of the cells. They can frequently be found floating in the mucilage.

In this case there can obviously be no doubt as to the true lysigenous origin of the mucilage-ducts (Fig. 10).

#### Method ii. Protogenetic Schizo-lysigenous Mucilage-canals.

In the petiole of *Angiopteris evecta* an alternative mode of development was noticed for many of the mucilage-ducts. These elements, to which both Brebner (2) and Lutz (14) had attributed a typical schizogenous development, were studied with special care.

The earliest stages agree in every respect with those described above under Method i, but the later stages follow a somewhat different course. The canal-initials, which arise very early, usually divide into 2, 4, or 6 with no obvious increase in size, and give rise to irregular groups of very small cells with dense cytoplasm and prominent nuclei (Fig. 1).

The contents of these small cells immediately begin to show signs of incipient mucilaginous degeneration. This brings about a decrease in the turgidity of the cells, which tend to round themselves off. At the same time small irregular areas are produced between adjacent cells. Special interest attaches to these areas, which are only slightly larger than ordinary intercellular spaces, since they give the staining reactions of mucilage.

It is quite possible, however, that the mucilaginous substance produced at this early stage is derived from the pectin of the middle lamella, which is probably the first part of the cell-wall to become disorganized. Such a condition indicates how typical schizogenous secretory cavities may have arisen. However, the latter are not known to occur in any recent group of Cryptogams.

Strictly speaking, therefore, these mucilage-canals have a schizogenous *origin*; on the other hand, it is certainly incorrect to say that they have a schizogenous *development*, since all subsequent increase in size of the cavity (together with the corresponding increase in volume of the mucilage) takes place by the rapid collapse of the surrounding cells.

It often happens that certain of the more peripheral cells of the group divide by walls radial to the centre of the canal; these cells usually retain their normal appearance for a considerable time after the innermost cells have completely lost their individuality. Thus the young mucilage-duct appears to possess a distinct epithelial layer (Figs. 2, 3, and 5).

But these so-called epithelial cells are very irregular in shape and size and have only a transitory existence. Moreover, they do not present the appearances generally associated with a living secretory epithelium.

There is no evidence to show that they have a secretory function; they all very soon break down, their disorganized remains helping to increase the contents of the duct. At a slightly later stage epithelial cells can no longer be detected lining the cavity of the duct, which now abuts directly upon the unmodified parenchymatous cells of the ground-tissue (Fig. 4).

The adult canals are irregularly distributed throughout the parenchyma of the petiole, in which they frequently branch and anastomose (Fig. 5).

### Method iii. Hysterogenetic<sup>1</sup> Lysigenous Mucilage-canals.

A distinct type of mucilage-duct was found in the adult petiole of *Kaulfussia aesculifolia* and in large roots of *Angiopteris evecta*.

<sup>1</sup> Frank, l. c.

These ducts appear subsequently in old mature tissues and are therefore hysterogenetic.<sup>1</sup>

In the young petiole of *Kaulfussia* numerous mucilage-ducts, which develop according to Method i, occur, whilst in the young roots of *Angiopteris evecta* no mucilage-ducts were observed.

This may explain why Van Tieghem (20) failed to find mucilage-ducts in the roots of this genus. But numerous mucilage-ducts in all stages of development were observed in sections through fairly old petioles of *Kaulfussia aesculifolia* and through adult roots of *Angiopteris evecta*. These elements were found in perfectly healthy petioles and roots, and the hypothesis that these canals are formed as the result of traumatic stimuli was proved to be untenable.

They arise by the collapse of groups of typical parenchymatous cells which are in other respects indistinguishable from the other cells which constitute the cortex.

At an early stage these cells can be distinguished by a decrease of turgidity which results in a slight decrease in size, whereby the intercellular spaces, which are typically rather large between the cells of the cortical parenchyma, exhibit a corresponding increase in size. But no signs of mucilage were observed in these intercellular spaces.

Meanwhile the cell-walls rapidly dissolve, while the cell contents become mucilaginous. The cells ultimately break down, and their disorganized remains coalesce to form the stringy mucilaginous contents of the cavity (Figs. 7 and 9).

As Newcombe (l. c.) pointed out, when lysigenous cavities arise subsequently to primary growth, there is generally a collapse but little or no tearing of cells.

The development of these peculiar mucilage-ducts seems to be very irregular both in time and space. The fully developed ducts often exhibit a striking appearance, for they may be very irregular in outline and occasionally attain large dimensions, as is shown in Fig. 9.

## 2. *Development and Structure of the Tannin-sacs and Tannin-ducts.*

Cells containing tannin are widely distributed throughout the sporophytic tissues of all six genera of the Marattiaceae.<sup>2</sup>

They are generally protogenetic and occur either singly or associated together in groups. When they occur singly they can easily be distinguished by their tanniniferous contents from the neighbouring cells, from which they may or may not differ in shape or size.

Their form varies in different regions of the same organ, but as

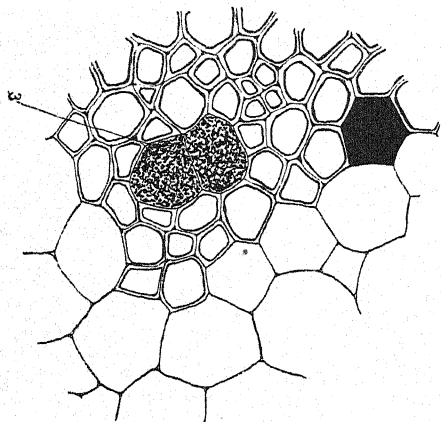
<sup>1</sup> Frank, l. c.

<sup>2</sup> The present writer's observations on *Kaulfussia* cannot be reconciled with the statement of Campbell (l. c., p. 185) that in this genus 'tannin-cells are practically entirely absent from the sporophyte throughout its whole existence'.

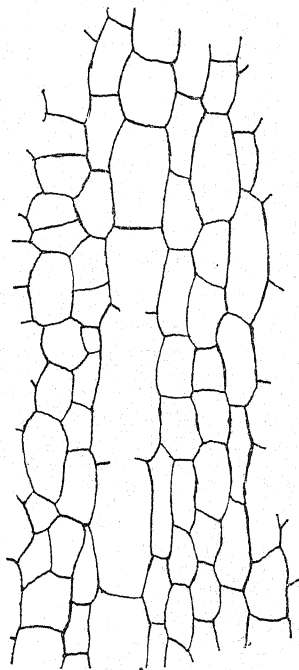


a general rule it was noticed that those associated with a vascular bundle are relatively much narrower than those which occur in the ground-tissue.

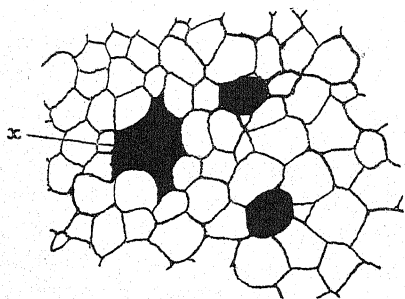
They invariably possess thin cellulose walls even when they are embedded in the fibrous zone of the petiole (Text-fig. 8).



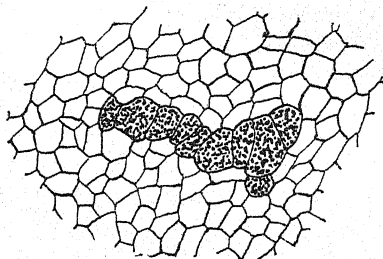
TEXT-FIG. 8. Tannin-cells in fibrous layer of petiole of *Angiopteris evecta*, Hoffm. *w.* = persistent parting-wall.  $\times 380$ .



TEXT-FIG. 10. Tannin-duct from the petiole of *Angiopteris evecta*, Hoffm. The contents of the duct are not shown.  $\times 220$ .



TEXT-FIG. 9. Tannin-cells in the petiole of *Angiopteris evecta*, Hoffm. The large tannin-cell *x* is crushed by the surrounding parenchyma.  $\times 220$ .



TEXT-FIG. 11. Series of tannin-cells from the stem of *Danaea nodosa*, Sm.  $\times 150$ .

They not infrequently occur associated together in regular series of superposed cells, e.g. in the stem, root, and petiole of *Angiopteris evecta* (Text-fig. 10; Fig. 5, *t.c.*); in the root of *Danaea alata*, *Danaea nodosa*, and

*Marattia attenuata*; and in the petiole of *Danaea simplicifolia*, *Marattia alata*, &c., &c.

They are less frequently associated together in irregular groups, e. g. in the stem of *Danaea nodosa* (Text-fig. 11), and in the sporangium wall of *Kaulfussia aesculifolia*, &c., &c.

It sometimes happens that the parting-walls of adjacent tannin-cells break down, the solution of the wall beginning in the centre and gradually spreading until a typical vessel or duct is formed, e. g. in the petiole and root of *Angiopteris evecta* (Text-fig. 10, and cf. Farmer, l. c., p. 269 and Pl. XV, Fig. 12), and in the root of *Marattia attenuata*. The breaking down of the parting-walls bears no relation to the age of the cells; it may take place either in very young or in fairly old tissues.

Such a series of stages in the development of a typical tannin-duct was described and illustrated by Lutz (l. c., pp. 134, 135; Pl. II, Figs. 3-7), who, however, mistook these tannin-ducts for lysigenous mucilage-canals, and stated that the tannin was eventually replaced by mucilage.

I was quite unable to confirm this explanation, since in all my material the tannin-cells (or ducts) are from the very beginning absolutely distinct, and, even in old specimens, bear no resemblance to the mucilage-canals. Whereas the mucilage-canals freely branch and anastomose (Fig. 5), the tannin-ducts seldom branch and never anastomose.

From our knowledge of the chemical composition of these secretions it would appear very improbable that one should be converted into the other; notwithstanding that in certain Angiosperms tannin occurs associated with mucilage.

In the stem of *Danaea nodosa* and in the sporangium wall of *Kaulfussia aesculifolia* irregular tannin-containing cavities are produced by the breaking down of the parting-walls between adjacent tannin-cells.

A true secretory epithelium is never present round the tannin-cells or tannin-ducts, but their contents often exert a considerable pressure upon the surrounding cells, which are slightly crushed and assume the appearance of an epithelium, to which, however, they bear only a superficial resemblance.

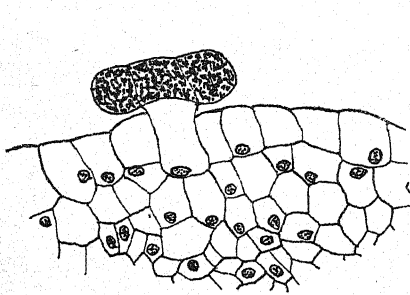
On the other hand, the tannin-cells (or ducts) are often compressed by the surrounding parenchyma (Text-fig. 9; Figs. 4 and 5).

Tannin-sacs are abundant in the ramenta of *Angiopteris evecta*, *Danaea nodosa*, *Danaea alata* (Text-figs. 12 and 13), *Marattia Cooperi*, and *Marattia fraxinea*; in the characteristic two-celled hairs of the petiole of *Kaulfussia aesculifolia* (Text-fig. 14); and in the paleae of *Archangiopteris Henryi*.

*Wound-tannin.* A copious secretion of tannin was observed in the immediate neighbourhood of wounds. In this respect the tannin may be compared with the secretion of resin which is induced by traumatic stimuli in certain Conifers.

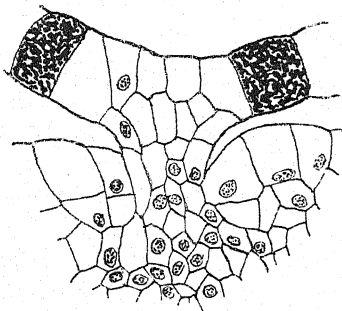
*Theoretical considerations.* In all the genera of Marattiaceae examined

by the present writer typical lysigenous mucilage-ducts were found. But in the petiole of *Angiopteris evecta* an alternative schizo-lysigenous development of the mucilage-ducts was observed. The cells lining the cavity of these ducts develop as an indefinite transitory epithelium. Thus it seems that, as Farmer and Hill (l.c., pp. 390-1) previously pointed out, the primitive method of mucilage-duct formation in this group of Ferns is

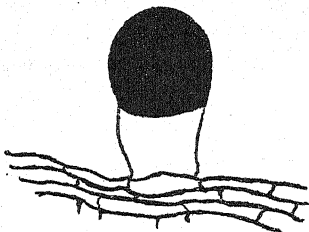


TEXT-FIG. 12.

Tannin-cells in ramenta of *Danaea alata*, Sm.  $\times 280$ .



TEXT-FIG. 13.



TEXT-FIG. 14. Tannin-cell in hair on petiole of *Kaulfussia aesculifolia*, Bl.  $\times 280$ .

lysigenous. The more advanced schizo-lysigenous method is restricted to the petiole of *Angiopteris evecta*.

It is generally acknowledged that this genus is the most specialized member of the Marattiaceae, and hence it is not surprising that in this genus alone a more complex mode of mucilage-duct formation obtains.

The form and position of the tannin-cells and tannin-ducts vary considerably even in different individuals of the same species, and afford no means of generic or specific distinction.

*Functions of the secretions.* Although the mucilaginous secretions are produced in the youngest parts of the plants, they do not disappear from the older parts, and consequently are lost to the plant when the leaves and their appendages are thrown off or when the stem and roots decay away.

It would thus appear that the mucilage constitutes a waste-product of the plant. The same explanation will serve for the tannin-sacs (and ducts), which also persist throughout the life of the plant organ in which they occur.

Both types of secretory tissue are equally abundant in the stem, leaves, and roots of these Ferns; it is therefore improbable that they are produced in connexion with a water-storing function, especially when it is remembered that all the representatives of this family are usually confined to localities where they would not be subjected to long periods of drought.

Whether these substances possess any biological significance in connexion with the attacks of animals or of parasitic Fungi it is impossible to say.

#### SUMMARY.

1. Lysigenous mucilage-canals were found in every genus and species of Marattiaceae examined. They are usually protogenetic, but occasionally hysterogenetic (e. g. petiole of *Kaulfussia aesculifolia*; root of *Angiopteris evecta*).

In the petiole of *Angiopteris evecta* the mucilage-canals may be lysigenous or schizo-lysigenous.

2. Tannin-cells are widely distributed through the sporophytic tissues of the Marattiaceae; they occur either as isolated tannin-sacs or grouped together in regular or irregular series.

Lysigenous tannin-ducts are formed by the solution of the septa between adjacent tannin-cells.

In conclusion, I desire to express my thanks to Professor J. B. Farmer, F.R.S., for much helpful advice and criticism.

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## EXPLANATION OF PLATE XVIII.

Illustrating Mr. West's paper on the Structure and Development of the Secretory  
Tissues of the Marattiaceae.

Figs. 1, 2, and 3. Successive stages in the development of mucilage-canals. In Fig. 3 the transitory epithelium is shown. From longitudinal sections of a young petiole of *Angiopteris evecta*, Hoffm. × 280.

Fig. 4. Part of a transverse section of an older petiole of *Angiopteris evecta*, Hoffm., showing four adult mucilage-canals in transverse section. Note complete absence of epithelial cells. *t.c.* = tannin cells. × 100.

Fig. 5. Part of a longitudinal section of a young petiole of *Angiopteris evecta*, Hoffm., showing early stage in development of a mucilage-canal. *t.c.* = tannin cells. × 60.

Fig. 6. Early stage in the development of a mucilage-canal. The protoplasm of many of the small cells is undergoing mucilaginous degeneration; at *x* the cell-walls have disappeared. From a transverse section of a young petiole of *Danaea nodosa*, Sm. × 280.

Fig. 7. Part of a transverse section of an old root of *Angiopteris evecta*, Hoffm., showing early stage in the formation of a mucilage-canal by the mucilaginous degeneration of part of the cortical parenchyma. *m.* = mucilage. × 280.

Fig. 8. Part of a longitudinal section through the apical region of a large root of *Marattia Cooperi*, Mre., showing a young mucilage-canal. Note absence of epithelial cells. *m.* = mucilage; *n.* = nuclei of disorganized cells floating in the mucilage. × 100.

Fig. 9. Part of a transverse section of an adult petiole of *Kaulfussia aesculifolia*, Bl., showing a large irregular mucilage-canal formed by the mucilaginous degeneration of typical parenchymatous cells of the ground-tissue. *m.c.* = mucilage-canal of the usual type. × 100.

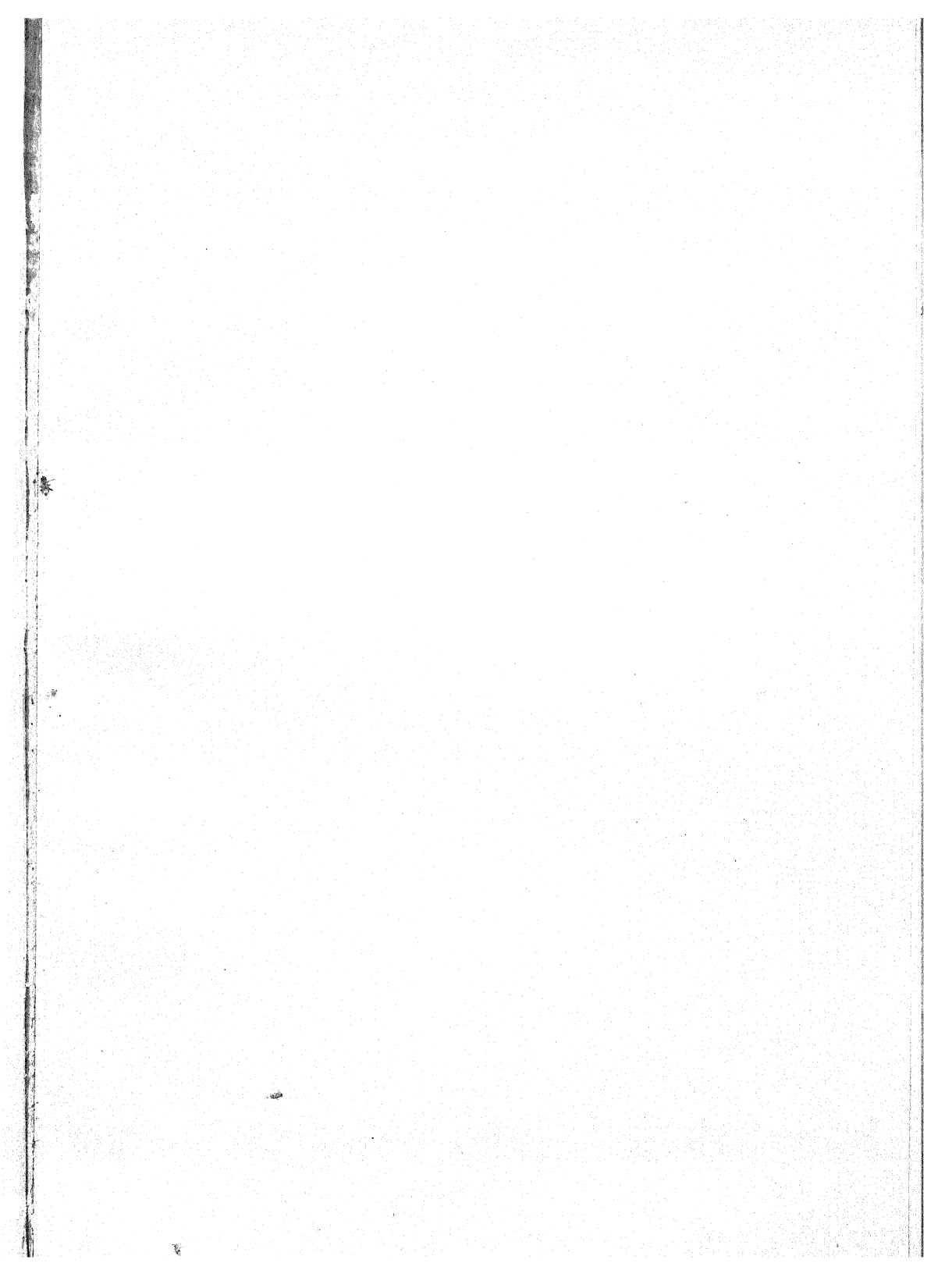
Fig. 10. Early stage in the development of a mucilage-canal from a single row of canal-initials. From a longitudinal section through the apical region of a large root of *Marattia Cooperi*, Mre. × 100.

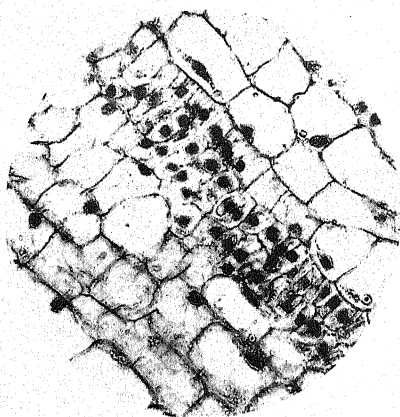
Fig. 11. Part of a transverse section of a large root of *Danaea alata*, Sm., showing early stages in the development of mucilage-canals. *t.c.* = tannin-cell. × 100.

Fig. 12. Early stage in the development of a mucilage-canal. The protoplasm of many of the small cells has undergone mucilaginous degeneration, while several of the cell-walls have already disappeared. From a transverse section of a young petiole of *Marattia Cooperi*, Mre. × 280.

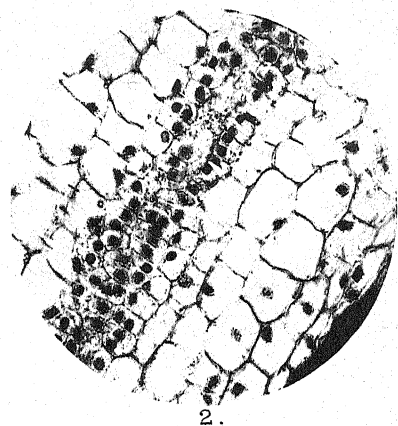
Fig. 13. Part of a longitudinal section through the apical region of a large root of *Danaea nodosa*, Sm., showing early stage in the development of a mucilage-canal. × 280.

Fig. 14. Early stage in the development of a mucilage-canal. The protoplasm of several of the small cells has undergone mucilaginous degeneration; at *x* the cell-walls have completely disappeared. From a longitudinal section of a young petiole of *Kaulfussia aesculifolia*, Bl. × 280.

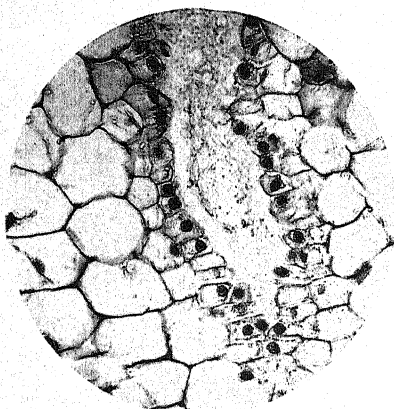




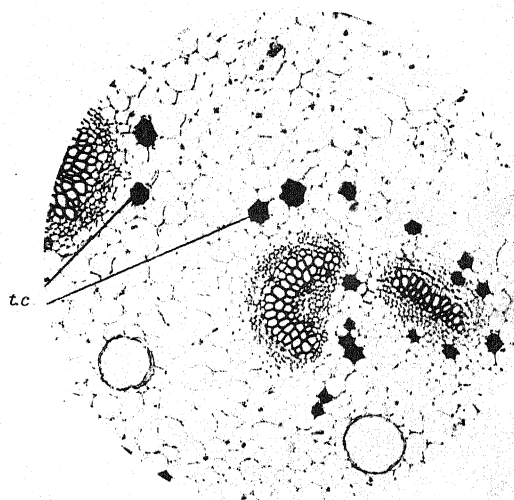
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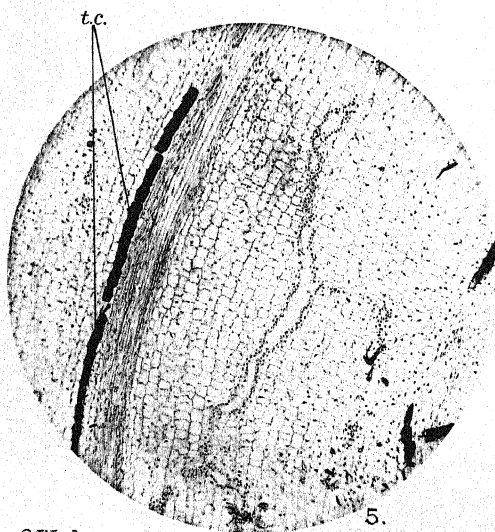
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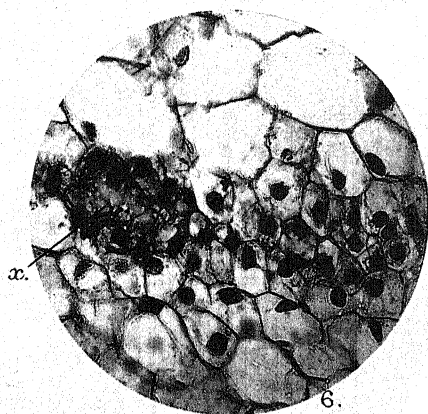
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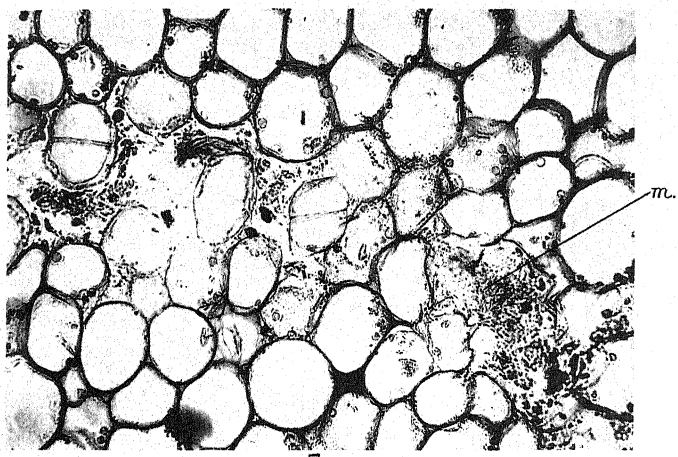


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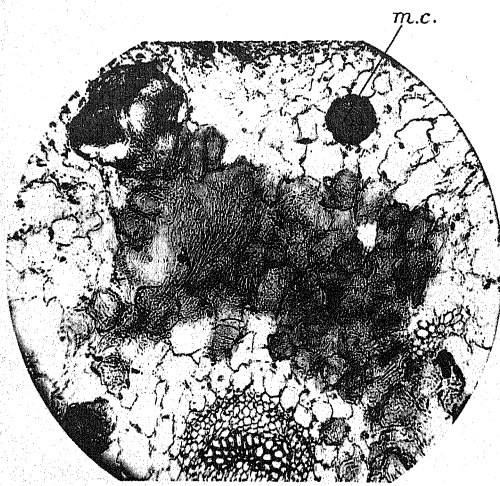




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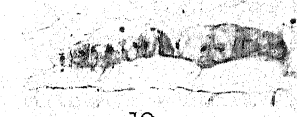
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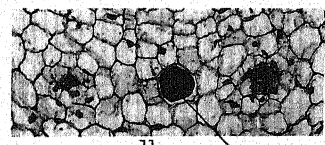
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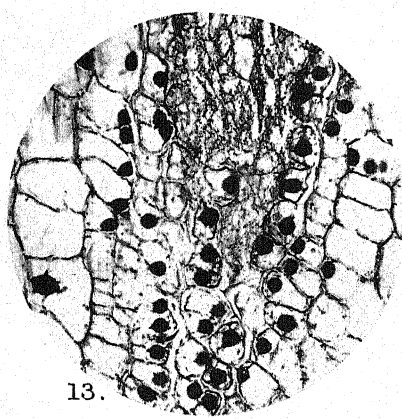
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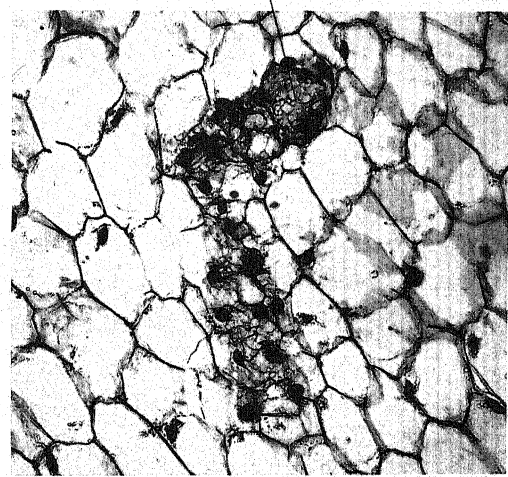
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# On *Glaucocystis Nostochinearum*, Itzigsohn.

BY

B. MILLARD GRIFFITHS, M.Sc.

With Plate XIX.

*GLAUCOCYSTIS NOSTOCHINEARUM*, Itzigs., is a unicellular solitary alga found generally in *Sphagnum*-bogs. It is ellipsoidal in form and measures from 30 to 45  $\mu$  in length, and from 18 to 25  $\mu$  in breadth. It has a chromoplast consisting of a number of strongly recurved radiating bands of a blue green colour. It reproduces by the formation of two, four, or eight daughter-cells within the mother-cell.

The systematic position of the organism has been doubtful. Chodat placed it in the Protococcaceae owing to its resemblance to *Oocystis* (*vide* Oltmanns, '04). Oltmanns can find no certain place for it, but refuses to put it in the Protococcaceae owing to its blue-green colour. West ('04) classifies it among the Cyanophyceae (or Myxophyceae). He divides the whole group into Glaucocystideae and Archiplastideae, and therefore places *Glaucocystis* outside the rest of the Cyanophyceae. The difficulty of assigning it to a definite group is intensified by conflicting evidence regarding its nucleus. Lagerheim states that the nucleus is merely a vacuole (Hieronymus, '92). Hieronymus describes a perfectly definite nucleus containing a body resembling a nucleolus.

I found the organism in considerable quantities at the beginning of the year 1910, and with this material at my disposal have carefully examined the nucleus with a view to discovering its exact nature. The investigation was suggested by Professor G. S. West in order to obtain further information concerning its cytology with a view to determining the systematic position of the organism. The results show that *Glaucocystis* is one of the Cyanophyceae, but with a nuclear structure of much greater definiteness than is found in the rest of the group, and possessing many features that make it almost comparable with the more highly elaborated nucleus of higher plants.

The organism occurred in a small permanent surface-water pool near Kidderminster, Worcs., among the lower portions of a dense growth of *Fontinalis antipyretica*. It was found at all seasons of the year, but most plentifully in the colder months. The pool was not more than a few

inches deep and about twenty square yards in area. The soil was a red marl. A dense vegetation of *Fucus communis*, *Alisma Plantago*, and *Ranunculus aquatilis* nearly filled the pool. The occurrence of *Glaucocystis* in other than a *Sphagnum*-bog is not by any means a usual thing. No *Sphagnum* is found in the immediate district.

#### COLLECTION AND FIXATION.

Tufts of the *Fontinalis* were pulled up and the water was squeezed out into glass tubes. Subsequently the sediment, consisting of decayed vegetable matter, filamentous Cyanophyceae, a few Desmids, Bacillarieae, and *Glaucocystis*, was fixed either in a  $2\frac{1}{2}$  per cent. solution of formic aldehyde, or in a mixture of three parts absolute alcohol and one part glacial acetic acid. One drop of sediment contained under favourable conditions as many as twenty specimens of *Glaucocystis*. It was not found possible to separate the organisms from the flocculent matter in which they were found. In order to facilitate the treatment of the sediment in the alcohols and staining reagents, a centrifuge was used to bring the material down quickly to the bottom of the tube.

#### STAINING REAGENTS.

For staining the chromoplast, safranin and fuchsin were found to be the best. For the structure of the nucleus, haematoxylin and gentian violet were most successful. Iron alum and haematoxylin proved fairly satisfactory also. In all cases the sediment was taken up through the alcohols into xylol, and mounted finally in Canada Balsam for examination.

#### THE CELL-WALL.

The cell-wall of *Glaucocystis* strikingly resembles that of *Oocystis*. The cell is ellipsoidal in shape and has apical thickenings on the inner side of the cell-wall. In addition, however, there is an equatorial thickening on the outer side of the cell-wall. The wall is fairly thick, but when in the mother-cell condition it becomes very thin and ultimately ruptures. When treated with iodine in potassium iodide and strong sulphuric acid the cell-wall turns blue. It consists, therefore, very largely of cellulose. In this respect the cell-wall of *Glaucocystis* differs from that of most Cyanophyceae.

#### THE CHROMATOPHORES.

These are of very remarkable form. Hieronymus ('92) describes and figures a series of radiating, strongly curved chromatophores from twelve to twenty in number, and of a blue-green colour. When the cell is about to divide, these chromatophores break up into a large number of oval plastids. After cell-division the chromatophores are re-formed. I made

regular monthly collections of *Glaucocystis* from the pool throughout 1913, and at frequent intervals between 1910 and 1913, but almost all the specimens found were in the division stage of the chromatophores. In only a few cases were the radiating bands seen. The unusual situation in which the organisms were found may be correlated with this. The usual habit is in *Sphagnum*-bogs, and in such places the chromatophores frequently show the structure described by Hieronymus. The colouring matter in the chromatophores consists largely of phycocyanin. In one case some specimens had been allowed to become unhealthy through long keeping in a tube. The phycocyanin came out of the plastids and filled the cell-sap with the characteristic blue colour.

#### THE CYTOPLASM.

This consists of a reticulum with granular threads. The size of the alveoli varies from 1 to 2  $\mu$ . The cytoplasm fills the cell completely and does not contain a central vacuole. On division of the cell, the cytoplasm constricts slightly in the equatorial plane, but the complete division takes place by a rectilinear fission transverse to the axis of the cell. In this respect *Glaucocystis* resembles many other Cyanophyceae, more especially the Chroococcaceae. The preliminary slight constriction of the cytoplasm does not seem to be correlated directly with the phases of nuclear division, but may occur either before or after. The final transverse fission takes place after nuclear division and is very rapid. The two parts of the cytoplasm round off into oval masses and daughter cell-walls are formed.

#### THE NUCLEUS AND NUCLEAR DIVISION.

The nucleus, both in structure and in its division stages, shows many features of a remarkable kind. At one stage it bears a striking resemblance to the nucleus of higher plants. In another stage it appears to be little more than a vacuole. The following phases may be observed:

(a) A large round space 11  $\mu$  in diameter lies in the equatorial portion of the cytoplasm, but close to the cell-wall. It stains very feebly indeed (Pl. XIX, Fig. 1). The surrounding cytoplasm also stains feebly, but it is full of small oval and round bodies which stain deeply. None of these deeply staining bodies are to be found immediately between the clear space and the wall of the cell. The clear space does not possess a membrane, but is simply a space free from staining bodies. It appears to be continuous with the cytoplasm, and is not a vacuole. Under moderately great magnification it certainly does look like a vacuole, but under high magnification it is seen to consist of the same extremely delicate reticulum as the cytoplasm. The boundary of the clear space is not perfectly regular, but only approximately spherical owing to the slight intrusion of the staining bodies on its extreme

outer edge. The deeply staining bodies in the cytoplasm lie at the intersection of the threads of the reticulum. The threads are very delicate and not granular at this stage.

(b) The clear space, which may be denoted the 'karyoplasmic area', now begins to contract and to move towards the centre of the cell. It becomes more deeply stainable, the staining being diffuse. Its reticulum, previously extremely delicate and difficult to see, becomes coarser and more visible. At the same time, the number of darkly staining bodies in the cytoplasm diminishes, and the whole cytoplasm becomes diffusely stainable. The karyoplasmic area does not possess a definite membrane at this stage (Fig. 2).

(c) The karyoplasmic area contracts further until it reaches a diameter of 6 or 7  $\mu$ , and a well-marked membrane forms. The cell as a whole increases in size also. The threads of the karyoplasmic reticulum become thick and stain more deeply. Deeply staining granules of chromatin appear at the intersections of the threads (Fig. 3). One granule becomes very much larger than the rest. It is circular or roughly polyhedral in outline. It is not homogeneous in structure, but is composed of many granules fused together. The dark bodies of the cytoplasm disappear completely, and the threads of the cytoplasmic reticulum become finely granular. These granulations do not stain deeply (Fig. 4). At this stage, the cell bears a striking resemblance to the cell of higher plants. There is a definite nucleus bounded by a membrane, and possessing darkly staining granules of chromatin. The large karyosome, roughly circular in form, resembles a nucleolus in position, but, as will be seen later, its behaviour is quite different from that of the nucleolus of higher plants.

(d) The concentration and definition of the nucleus is a preparation for division. The nucleus elongates to a length of about 11  $\mu$ , but the diameter remains about the same (Fig. 5). The nucleolus-like karyosome becomes a very conspicuous object. It grows very large, probably by the addition of the smaller granules of chromatin previously scattered on the nuclear reticulum. The nuclear reticulum itself ceases to be visible, and the nucleus becomes uniformly diffusely stainable, with the large karyosome lying in it. The karyosome now divides by a transverse fission into two equal parts semicircular in outline, which separate a short distance. No case was observed among the very large number of dividing nuclei in which there was anything in the nature of a polar separation of chromatin substance or the formation of a chromatic figure. In every instance the process consisted of the aggregation of chromatic material into a large karyosome, which subsequently divided by simple transverse fission. After the division of the karyosome, the nucleus divides in a precisely similar manner (Figs. 6 and 8). A large number of specimens were in this stage. Each half of the nucleus is semicircular or semi-elliptical, with a very distinct

straight edge along the line of fission. Sometimes the half-nucleus has one or more pointed processes (Figs. 8 and 9). These always lie on a line radiating from the karyosome towards the line of transverse fission. In some cases, numerous radiating lines of strain were found traversing the whole cytoplasm. Their centre of convergence lies at the karyosome, and they do not appear to cross the line of transverse fission of the cytoplasm (Fig. 8). The cytoplasm may show signs of division even before the division of the nucleus is initiated. On the other hand, it may be delayed until after. A distinct constriction appears in the equatorial plane. This is followed by a straight transverse fission which is always delayed until the nucleus has divided. After this, the cytoplasm rapidly rounds off, each half-nucleus becomes spherical, and two daughter cell-walls are formed (Fig. 10). In some cases the nucleus divides into four (Fig. 11), or even into eight (Figs. 12 and 14), and four or eight daughter-cells are formed simultaneously. In other cases two or four daughter-cells are formed, and a second nuclear division takes place (Fig. 15). Previous to the formation of daughter-cells, the mother-cell increases greatly in size. In the resting condition the cell measures about  $30\ \mu$  by  $18\ \mu$ . During division it may attain a size of  $45\ \mu$  by  $25\ \mu$ , that is, it about doubles in volume. The daughter-cells are therefore about normal size. In this increase in size before division *Glaucocystis* differs altogether from *Oocystis*. Bohlin ('97) records that *G. cingulata* also varies greatly in size, the diameter varying from 12 to  $56\ \mu$ , and the length from 16 to  $56\ \mu$ .

(e) Division having taken place and the daughter-cells having been formed, the nucleus of each begins to undergo a series of degradations. The karyosome, that played so important a part in cell-division, breaks up. The stainable nuclear reticulum disappears, and the nuclear membrane can no longer be seen. The cytoplasm once more becomes full of deeply staining granules, and the karyoplasmic area less definite and more feebly stainable. Ultimately the cell reaches the resting condition described in the first section. The nucleus is now represented by the vacuole-like karyoplasmic area, and ceases to resemble the nucleus of higher plants. The chromoplast, which had broken up into numerous oval plastids during the stages of cell-division, reorganizes, and becomes once more a series of radiating recurved bands.

#### COMPARISON WITH OTHER FORMS.

The nucleus of the Myxophyceae is characterized by its irregular form, the absence of a nuclear membrane, the absence of a nucleolus, and by a tendency for cell-division to take place independently of nuclear division. Chromatin is present, and Kohl ('03) and Hegler have observed the formation of a chromatic figure. Wager ('01) also states that owing to the drawing out of the chromatin threads elongated cells often show stages

resembling true karyokinetic division. Darkly staining granules in the cytoplasm have been recorded under the names of central granules, metachromatin, and volutin, and their gradual disappearance by diffusion has been noted (Guilliermond ('12), Minchin ('12)).

Miss Acton ('14) has described the nucleus of *Chroococcus*. In *C. macrococcus* it is confined to a definite area, and is more or less oval in form. It contains chromatin, but has no nuclear membrane or nucleolus. It divides by simple transverse fission. In other species the nucleus is of the 'open' type. In *Merismopedia elegans* no nucleus is present in certain stages, but later on granules of metachromatin appear in the cytoplasm, followed by the formation of a deeper staining nuclear area. The latter develops darkly staining granules, while the metachromatin disappears from the cytoplasm. The nucleus divides, apparently by a transverse fission, and subsequently degrades and disappears. The cytoplasm commences to constrict before actual nuclear division, and the final division is completed after the division of the nucleus.

The cytology of *Glaucocystis* shows a more highly elaborated nuclear structure than any other member of the Cyanophyceae, but nevertheless it is only a difference in degree and not in kind. In stage (a) described above, the nucleus is apparently of the 'open' type. One might say that the karyoplasmic area is only potentially a nucleus. It is continuous with the rest of the cell protoplasm and devoid of stainable material. It is only distinguished from the general protoplasm by the marked absence of the comparatively large granules of metachromatin. The area is not perfectly definite, and is not bounded by a membrane. It is not in any way a vacuole. In this stage it is comparable with the 'open' nuclei of many of the Hormogoniae, except that it is of very regular form.

In stage (b) the karyoplasmic area moves to the centre of the cell and diminishes in size. The metachromatin granules of the cytoplasm diminish in number. At the same time the nuclear area, although without signs of a stainable reticulum, is diffusely stainable as a whole. It seems that the metachromatic substance is being taken into the nuclear areas in a diffuse form. Similar diffusion of metachromatin has been noted in other cases by Acton ('14), by Wager and Peniston ('10), and by Lutman ('11).

In stage (c) the nucleus has contracted to its smallest size. A karyoplasmic reticulum of thicker threads has developed. This stains deeply, probably owing to minute granules of chromatin in the threads. At the intersection of the threads larger chromatin grains are seen. With haematoxylin these grains take a dull dark red colour. They seem to be solid. The metachromatin grains of the cytoplasm are coloured a bright purple-red, and appear to be translucent. By this time all the metachromatin has disappeared from the cytoplasm. The cytoplasmic reticulum consists of granular threads. At this stage, therefore, the cell structure resembles that

of *Chroococcus macrococcus*, or of *Merismopedia elegans* in its stage preparatory to division. *Glaucocystis*, however, shows a more elaborate structure in two ways, first in the possession of a definite nuclear membrane, and secondly in the formation of the very large mass of chromatin resembling a nucleolus in position, which I have referred to as the 'large karyosome'.

In stage (*d*) the behaviour of the nucleus is different from any other of the Cyanophyceae. The large karyosome gradually attains a considerable size by drawing to itself all, or nearly all, the chromatin of the nucleus. Nuclear division is initiated by the transverse fission of the karyosome, and is completed by a similar division of the nucleus. This division is shared in by the whole cell. The cytoplasm shows strain lines radiating from the two karyosomes in some cases. Similar phenomena have been observed in *Lyngbya* (Brown, '11). Although the cytoplasm shows at first the Myxophycean character of division independent of the nucleus, it waits until nuclear division is complete before completing its transverse fission. This transverse fission is exhibited by such forms as *Chroococcus macrococcus* and *Merismopedia elegans*, but their nuclei remain in the stage of the simple chromatin reticulum, and there is no sign of the aggregation of chromatin material for purposes of equal division. In this respect *Glaucocystis* appears to have evolved a rough process of chromatin distribution comparable with the karyokinesis of high plants. The karyosome would be, according to this view, rather of the nature of a chromosome than of a nucleolus.

The organism shows also a higher specialization in its formation of daughter-cells. Alone among the Cyanophyceae, it produces daughter-cells similar in outward form to those of the mother-cell and within the mother-cell wall (as in *Oocystis*). There is, however, a profound difference, as the mother-cell of *Glaucocystis* nearly doubles in volume before dividing, whereas in *Oocystis* this is not the case.

In stage (*e*) the nucleus has finished its work of division and proceeds to take to pieces the structure it had elaborated. The large chromatin karyosome breaks up, the nuclear reticulum disappears, and once more the brilliantly staining metachromatin granules appear in the cytoplasm. The nuclear area increases in size, moves towards the side of the cell, and assumes the clear vacuole-like state of the resting condition.

*Glaucocystis* shows, therefore, a near approach to the cytological structure of higher plant cells, both in its actual nucleus and in the formation of daughter-cells. In higher plants the elaborate structure of the nucleus is retained permanently, but in this organism the nucleus reverts to the 'open' condition, and for each division must reconstruct itself.

That *Glaucocystis* is one of the Cyanophyceae is shown by the following characters: (1) The nucleus is of the 'open' type at one stage. (2) Nuclear division takes place by transverse fission, and the division of the cytoplasm



shows a distinct tendency to be independent of the division of the karyoplasm. (3) Phycocyanin is present as the colouring substance.

It differs from most Cyanophyceae in the following particulars: (1) The nucleus preparatory to division becomes 'closed'. It has a membrane and a nuclear reticulum possessing chromatin. The chromatin is aggregated into a single mass which divides by transverse fission. (2) After division of the cytoplasm, the two parts become rounded off and daughter-cells are formed, very similar to those of *Oocystis*. (3) The cell-wall consists very largely of cellulose. (4) There is a definite and elaborate chromoplast.

#### SUMMARY.

*Glaucocystis Nostochinearum*, Itzigsohn, is a unicellular solitary alga found generally in *Sphagnum*-bogs. It is ellipsoidal, and measures from 30 to 45  $\mu$  in length, and from 18 to 25  $\mu$  in breadth. It has a small internal polar thickening of the cell-wall at each end, and an equatorial external thickening. The cell-wall is composed mainly of cellulose. There is a definite chromoplast consisting of a number of strongly recurved and radiating bands of a blue-green colour. These break up into numerous short pieces in the division stage of the cell. The organism reproduces by the formation of two, four, or eight daughter-cells lying freely within the mother-cell wall.

In the resting stage the nucleus is of the 'open' type. It consists of a large colourless spherical portion of the delicate reticulate protoplasm, and is practically unstainable. It is only distinguished from the general cytoplasm by the complete absence of the metachromatin granules. This portion lies close against the cell-wall.

In the division stage, the nuclear portion of the protoplasm contracts, becomes coarsely reticulate, moves to the centre of the cell, and becomes stainable. Chromatin develops in this reticulum, and a nuclear membrane forms. The metachromatin granules of the cytoplasm gradually disappear. The chromatin of the nucleus aggregates into a large karyosome. This divides by transverse fission, and each part rounds off. The cytoplasm divides by transverse fission, each half rounds off, and daughter-cell walls are formed.

*Glaucocystis* is probably a member of the Cyanophyceae owing to the presence of an 'open' nucleus at one stage; the tendency of cytoplasmic division to take place independently of nuclear division; and to the presence of phycocyanin in the chromoplast. The very high differentiation of the nucleus in the dividing stage; the elaborate chromoplast to which the phycocyanin is confined; the formation of daughter-cells very similar to those of *Oocystis*; and the cellulose character of the cell-wall, are features

which separate *Glaucocystis* from all the rest of the Cyanophyceae, and probably justify it being placed in an entirely separate group of that division.

BOTANICAL LABORATORY,  
UNIVERSITY OF BELFAST,  
December, 1914.

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## DESCRIPTION OF PLATE XIX.

Illustrating Mr. Griffiths's paper on *Glaucocystis Nostochinearum*, Itzigsohn.

All drawings (except Fig. 12) were made with a Zeiss Abbé camera-lucida on a Zeiss microscope with achromatic objectives and compensated oculars. For finest details an oil-immersion  $\frac{1}{2}$  objective was used. Diffuse staining is indicated by fine dots. The cell-wall is not shown in many cases.

Fig. 1. *Glaucocystis Nostochinearum* in resting condition.  $\times 857$ . *k.*, karyoplasmic area; *m.*, cytoplasm full of metachromatin granules.

Fig. 2. Cell with karyoplasmic area in centre.  $\times 857$ . *e.*, equatorial thickening; *p.*, polar thickening; other letters as before.

Fig. 3. Cell with definite nucleus.  $\times 857$ .

Fig. 4. Cell with definite nucleus.  $\times 857$ . *l.k.*, large karyosome of chromatin.

Fig. 5. Cell with elongated nucleus.  $\times 857$ .

Fig. 6. Cell with nucleus in process of division by transverse fission.  $\times 857$ . The large karyosome has already divided.

Fig. 7. Cell with nucleus completely divided.

Fig. 8. Cell with divided nucleus, one half of which has a process.  $\times 857$ . In the cytoplasm are 'strain lines', radiating from the two half-nuclei.

Fig. 9. Cell with divided nucleus, each half with processes.  $\times 857$ .

Fig. 10. Mother-cell with two daughter-cells.  $\times 545$ .

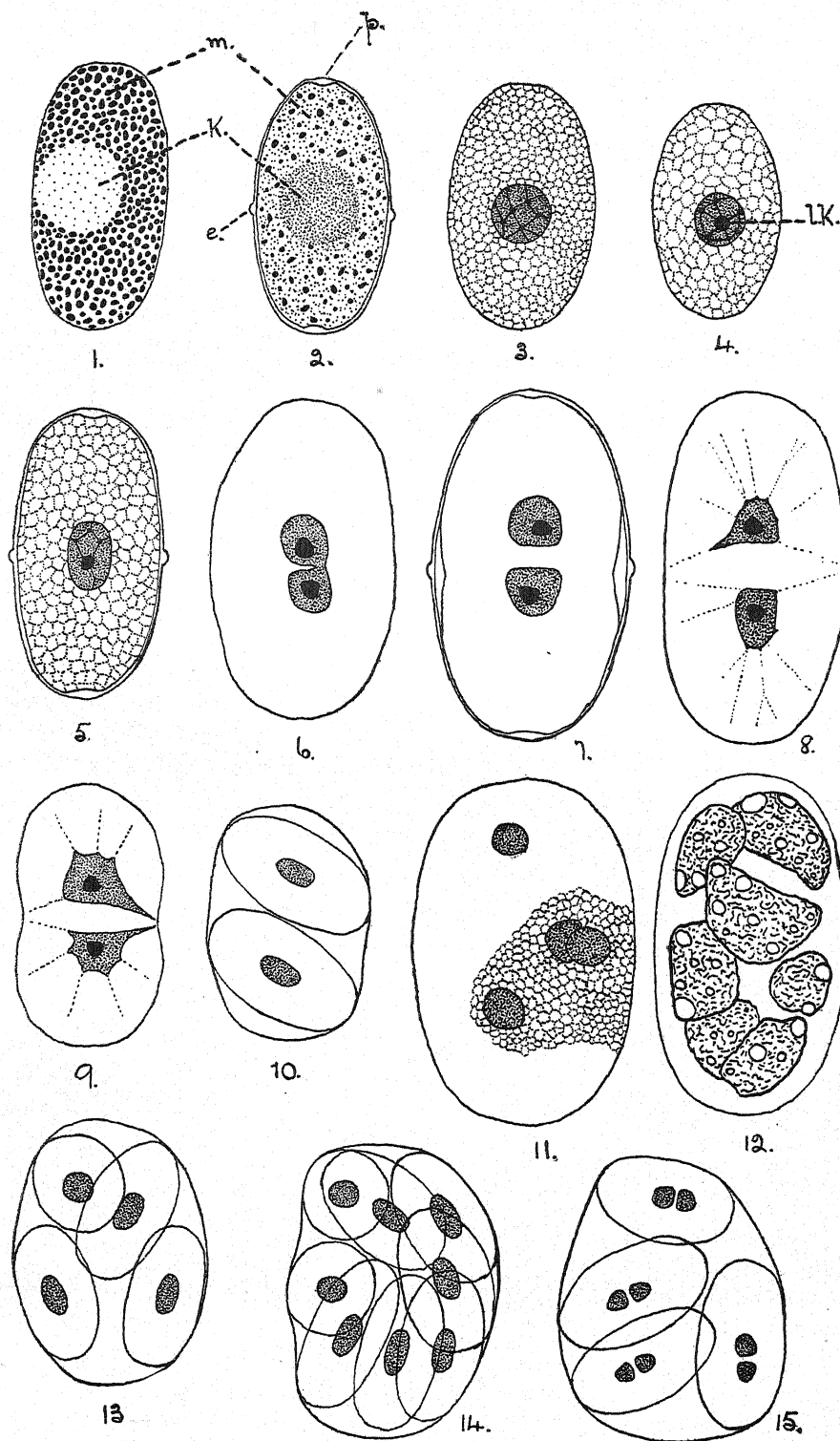
Fig. 11. Cell with nucleus divided directly into four.  $\times 857$ .

Fig. 12. Unhealthy cell with phycocyanin diffused in cell-sap.  $\times 857$  approx. The protoplasm has divided into apparently seven, but possibly eight, portions. The nucleus has probably divided into eight in this case.

Fig. 13. Mother-cell with four daughter-cells.  $\times 545$ .

Fig. 14. Mother-cell with eight daughter-cells.  $\times 545$ .

Fig. 15. Mother-cell with four daughter-cells.  $\times 545$ . Each daughter-cell has undergone subsequent nuclear division, but cytoplasmic division has not yet taken place. Several cases of two daughter-cells were observed in which a second nuclear division was taking place.



GRIFFITHS—GLAUCOCYSTIS NOSTOCHINEARUM, ITZIGS.

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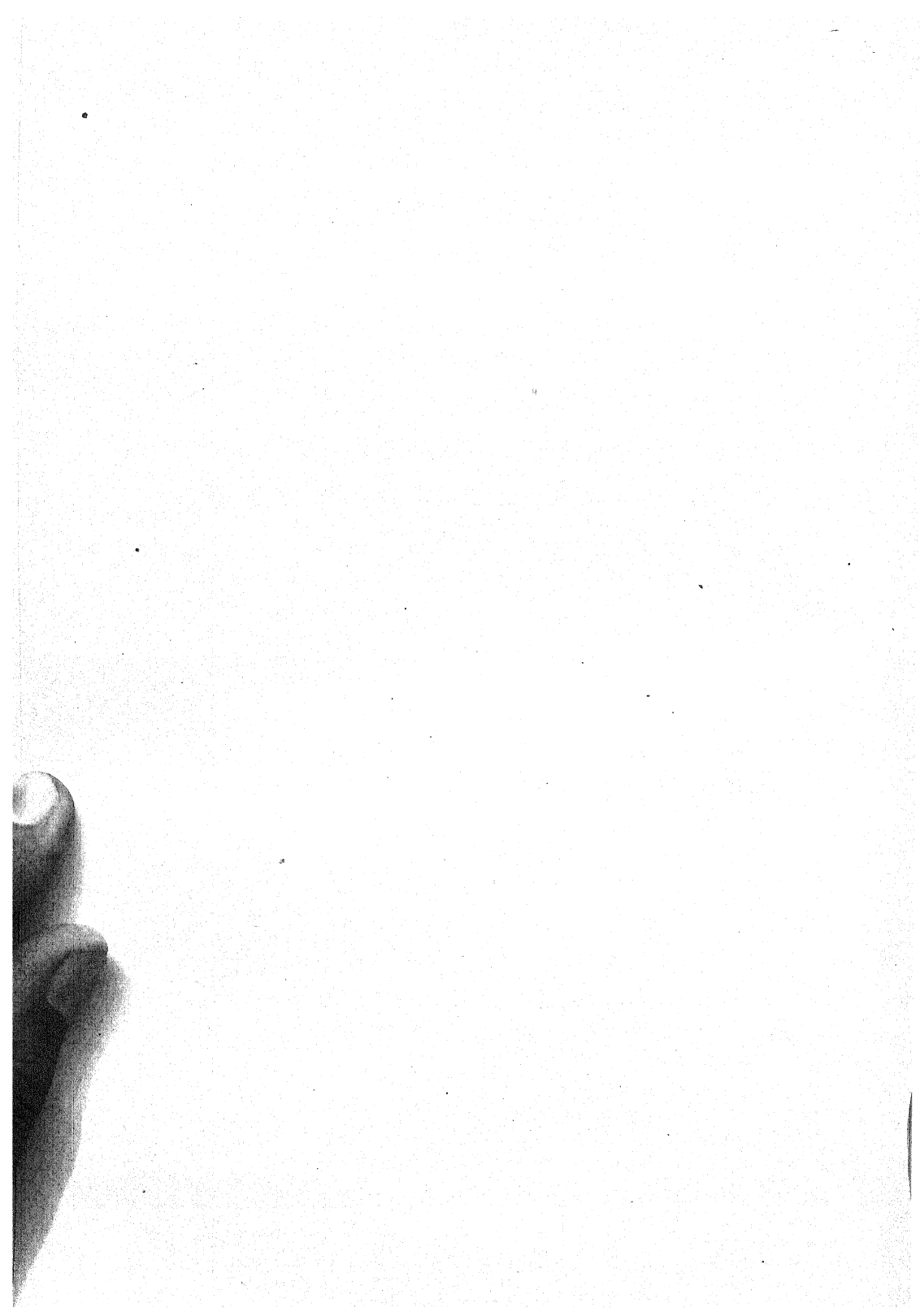
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# Sex Determination in *Mnium hornum*.

BY

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With Plate XX.

THE method of sex determination in dioecious plants has recently been the subject of a number of investigations, and, amongst these, those published by Él. and Ém. Marchal on the Apospory and Sexuality of the Mosses have created very considerable interest.

In a paper which appeared in 1906 (9), these investigators showed experimentally that, in the case of dioecious mosses, the spores produced in a single sporogonium are heterogeneous as regards their sexual characters; the spores are unisexual, the male giving rise to protonemata which bear exclusively male axes while the female produce protonemata which develop female axes only. They also proved that the protonema obtained vegetatively from the gametophyte always retains the sex of the parent plant. In protonemata produced either from spores or vegetatively from the gametophyte the sex is constant, and is not affected by external conditions.

In 1907 (10), a further paper was published dealing with the sexual characters of protonemata produced aposporously from the sporophyte of dioecious mosses. It was discovered that such protonemata always gave rise to a certain proportion of hermaphrodite axes, and it was presumed that no meiotic phase had taken place prior to the production of these individuals. The writer therefore concluded that, in the normal life-history of these mosses, the sex of the spores is determined at the reduction division immediately preceding their formation.

A third contribution appeared in 1909 (11), and in this the writers showed that, although the plants produced aposporously develop sexual organs, these are sterile and no sporogonia are produced. In this paper the discovery of organs of mixed sex is recorded in plants of *Bryum caespitium* and *Mnium hornum* which had been produced aposporously. The authors emphasized the fact that up to that time no mixed organs had ever been described in dioecious mosses; Hy (7) had previously observed similar organs in *Atrichum undulatum* and Holferty (6) in *Mnium cuspidatum*, but both these species are monoecious.

Organs of mixed sex had, however, been noted in two dioecious mosses previously to 1909. Lindberg in 1879 (8) described and figured such organs in *Brachythecium erythrorrhizon*. This moss is described as monoecious in the 'Bryologia Europaea' (4, 14), but Lindberg, after the examination of numerous specimens, concluded that this species is really dioecious. The mixed organs were discovered in heads situated on stems which also bore normal female inflorescences; some of the organs resembled antheridia and others archegonia, while a complete series of intermediate forms were also present. Bergevin in 1902 (1) discovered and figured similar structures in *Plagiothecium sylvaticum*, an undoubtedly dioecious species; among the examples described, some are monoecious, some synoecious, and organs showing all stages of transition between antheridia and archegonia are found; no sections of these organs were made. A similar instance has been discovered in *Mnium hornum* by the present writer.

The axis which bore the organs of mixed sex had the appearance of a male individual and was collected in Kent in the spring of 1911, with a number of others which, as far as they were examined, all bore normal antheridia. The specimen was preserved in Flemming's weak fluid, and was examined by means of longitudinal sections. A considerable number of normal antheridia are borne in the head, and the majority of these contain almost mature spermatozooids; no normal archegonia are present.

Unfortunately the whole of the sections were not retained, but in those kept fourteen organs of mixed sex were discovered. These show almost all transitions in structure between archegonia and antheridia. The organs represented in Pl. XX, Figs. 1, 2, 3, 4 resemble the normal female organ in form, consisting of a venter and elongated neck. The walls of the venter are, in most cases, only one cell in thickness, and thus differ from those of the normal archegonium; traces of a double wall are however seen in the organ represented in Fig. 4. The neck canal-cells have in many cases divided by walls parallel to the axis of the organ, and the resulting cells resemble spermatogenic cells; this is particularly the case in Fig. 1. It is, however, difficult to definitely ascertain the nature of these cells as, in most cases, they appear to have undergone partial degeneration; it seems evident that the organs under consideration possess archegonial characters, in so far that a ferment is produced by the neck-cells which acts upon the cells present in the canal and brings about their partial conversion into mucilaginous material. It is improbable that the condition of the cells in question is due to imperfect fixation as spermatogenic cells in neighbouring normal antheridia are well preserved. In the organ shown in Fig. 3 two cells are present in the venter, each containing a deeply-staining nucleus of medium size and somewhat scanty cytoplasm. It is probable that the upper of these represents the ventral canal-cell, and the lower the ovum. Similar cells are

found in the younger archegonium represented in Fig. 1. In the organ shown in Fig. 2 two naked cells are present in the venter, and the canal only contains a small quantity of mucilaginous material. In this case it appears probable that the contents of the neck have been ejected, leaving the ovum and ventral canal-cell in the cavity of the venter; unfortunately the upper part of this organ had been destroyed during manipulation. Although it is doubtful whether fertilization could have taken place in the structures represented in Figs. 1 and 3, there appears to be no reason why one of the cells present in the venter of the organ shown in Fig. 2 should not have functioned as an ovum. In Fig. 4 three cells are apparently present in the venter.

The organ shown in Fig. 5 has the general form and appearance of an antheridium, but differs in being longer and narrower than the normal organ. The upper part contains numerous spermatids, while in the central part of the lower portion two cells are present which bear a close resemblance to an ovum and ventral canal-cell, both in appearance and position. The spermatids closely resemble those found in the normal antheridia, and little or no production of mucilaginous material appears to have taken place. The organs previously described may perhaps be looked upon as modified archegonia, but that shown in Fig. 5 is obviously bisexual.

It has been already pointed out that É. and Ém. Marchal have discovered organs of mixed sex in aposporously produced plants of *Mnium hornum*, and it might be urged that the organs just described were borne on an individual which had been aposporously produced. It has been shown by Brizi (3) that capsules of *Funaria hygrometrica* can give rise to protone-mata while still attached to the living moss plant, and it is not improbable that apospory may sometimes take place in nature. É. and Ém. Marchal (12) have shown that a plant produced in this way would possess the diploid number of chromosomes. An examination of the normal antheridia was therefore made, and this led to the discovery of one in which divisions of the spermatogenic cells were taking place. The dividing cells are in the condition of late prophase, and there is little difficulty in determining the number of chromosomes (Figs. 6, 7, and 8). A number of counts were made, and in all cases six chromosomes were present. As it has been previously shown (18, 19) that this is the haploid number for *Mnium hornum*, it is evident that the plant in question did not have an aposporous origin.

In view of the discovery of organs of both sexes on a single axis of *Mnium hornum*, a further examination of the results obtained by É. and Ém. Marchal is rendered necessary. According to these investigators there is an absolute separation of the sexes at the reduction division. The unisexual character is retained throughout the haploid phase, and the reunion of the sex determinants is brought about by fertilization. 'La réduction chromatique . . . est, à coup sûr, la cause déterminante de la disjonction sexuelle. Le caractère unisexe de la spore conserve rigou-



reusement à travers tout la phase haploïdique. . . . L'acte de la fécondation réunit à nouveau dans l'œuf les deux déterminants sexuels' (10, p. 766). In an aposporously produced individual it was assumed that no previous reduction had taken place, and in a later paper (12) the correctness of this assumption is demonstrated, for it is shown that the gametophyte of an aposporously produced moss contains the  $2n$  number of chromosomes. In the absence of meiosis such an individual, according to the theory, will be necessarily bisexual, and proof that this is actually the case is brought forward by means of numerous cultures (10).

The proportion of bisexual axes in such cultures is, however, unexpectedly small. In the case of *Bryum caespitium*, out of 1,738 axes examined, 1,579 or 90.8% were found to be male, 154 or 8.8% bisexual, and 5 or 0.28% female. During the third month of the production of sexual organs the proportion of bisexual axes rose to 11.2% (10, p. 782). *Mnium hornum* and *Bryum argenteum* gave almost similar results.

If there is an absolute separation of sex determinants at the reduction division as suggested by É. l. and É. m. Marchal, it would be expected that at least a very large proportion of the axes formed on aposporously produced protonemata would bear both male and female organs. The results just quoted show, however, that this is not the case, and a further explanation must evidently be sought for to account for the great preponderance of male individuals. It is evident that the proportion is not affected by external conditions for, in cultures of dioecious mosses produced from spores, carried out by the same investigators (9) under similar cultural conditions, the numbers of male and female individuals were approximately equal; variations in light intensity, heat, and nutritive conditions had no appreciable effect on the proportions of the sexes.

É. l. and É. m. Marchal assume that the unisexuality of the plants in the aposporously produced cultures is only apparent, and that it hides a potential hermaphroditism. Evidence for this is brought forward by showing that, in the second aposporous generation, i. e. in plants borne on protonemata normally produced from the leaves of the first aposporous generation, a small number of synoecious axes is always found, and this proportion is approximately constant whatever the sexual condition of the parent plant.

The production of protonemata from various parts of the gametophyte may be looked upon as a special form of vegetative reproduction, and it would be expected that such protonemata would produce axes of similar sex to that of the parent. This, indeed, has been shown to be the case by É. l. and É. m. Marchal in normally produced individuals (9). In the case of *Bryum caespitium*, however, a protonema derived from an aposporously produced synoecious axis gave rise to twenty-two axes, of which eighteen or 81.8% were male and four or 18.2% bisexual. Male and female axes gave

similar results. It is noteworthy that the percentage of bisexual axes produced has no relation to the sexual condition of the parent; the protonema derived from a bisexual individual does not produce a larger number of bisexual axes than one produced from a male or female plant; in all cases a large proportion of male axes are produced.

Several instances of the occurrence of hermaphrodite axes in various dioecious mosses have been mentioned above, and these render it questionable whether the proportion of bisexual individuals in the aposporous generations is really higher than that occurring in normal plants. If, as the result of further research, it is established that the increased proportion does exist, the possibility that it is brought about by the disturbance in the metabolic processes caused by the abnormal number of chromosomes present in each cell must be considered. It has been shown by É. l. and É. m. Marchal (11) that the presence of the diploid number of chromosomes results in the increased size of the organs, cells, and nuclei of the aposporously produced plants, and it appears to be possible that it has also had a disturbing influence on the sexual condition of the individuals.

Strasburger (15) in discussing the results obtained by É. l. and É. m. Marchal points out that the work of Philibert is of importance. Philibert (13) found that in *Homalothecium fallax*, *Camptothecium lutescens*, and *Fissidens bryoides* protonemata derived from dying leaves and lower parts of the female plants produced small male plants. These mosses are normally dioecious, but it is obvious that in these cases no complete sex separation can have taken place. The peculiar distribution of sexual organs in *Mnium cinclidioides* described by Milde in 1865 (12a) must also be considered here. This species is normally dioecious, but in certain apparently sterile axes Milde found small bud-like structures each containing antheridia and archegonia. In this case also sex separation must therefore be incomplete.

If the conclusions of É. l. and É. m. Marchal are accepted, it must necessarily follow that in the Musci each kind of gamete bears only the potentialities of its own sex. There is, however, no direct evidence that this is the case in the Bryophyta. No instances of apogamy are at present known in this group, and investigations as to the sexual condition of particular species are very few in number. Apart from the work of É. l. and É. m. Marchal no researches of this kind have been carried out in the Musci. Among the Hepaticae *Sphaerocarpus* has, however, been the subject of a somewhat similar investigation. Strasburger (16) quotes the results obtained by Ch. Douin, who examined the sexual condition of the plants arising from the four spores of each tetrad in two species of this genus. In both *S. terrestris* and *S. californicus* the spores of each tetrad remain in contact, and the resulting plants are in consequence found in groups of four. Eighty-one of these groups were examined, and in sixty-four of these two male and two female plants were found; in thirteen cases

no conclusion could be arrived at on account of the failure of germination of some of the spores; in four groups the results did not agree with the supposition that two male and two female spores were present in the tetrad. Strasburger accepts this as proof that sex separation takes place at the division of the spore mother-cells, and results in the formation of two male and two female spores. Blakeslee (2) has investigated the sexual condition of the spores in *Marchantia polymorpha* and *Fegatella conica*, and finds that in each of these species the spores from any one capsule give rise to both male and female plants.

It is interesting to note that variations in the sexual condition of normally dioecious species have been discovered in the Hepaticae as well as in the Musci. In *Preissia commutata* the occurrence of an androgynous receptacle has been described by Townsend (17), and such organs are not infrequently found in this species. The investigations on the Hepaticae which have just been described are of the type of that of Él. and Ém. Marchal on the Musci, and do not bring forward any direct evidence as to the sexual condition of the gametes.

Investigations carried out on other groups are more numerous in number, but it is questionable how far the results obtained are applicable to the Bryophyta.

Strasburger fully discussed the question of sex determination in 1909 (16), and concluded that in plants generally each kind of gamete bears only the tendency of its own particular sex, i. e. maleness is confined to the spermatozoid, and femaleness to the ovum. Correns (5), on the other hand, concludes that in the higher plants and animals the germ-cells of one sex are homogametic, while those of the other are heterogametic; he considers that it is probable that the homogametic germ-cells agree in sexual tendency with the sex producing them, while of the heterogametic germ-cells half bear the tendency of one sex and half of the other. It appears, therefore, that considerable difference of opinion exists on this point, and that the evidence is not by any means conclusive.

The results obtained by Él. and Ém. Marchal in their experiments with the Musci are of great interest, and emphasize the necessity for further research. In view, however, of the occurrence of mixed organs in an axis of *Mnium hornum* which has been just described, and the similar cases previously noted by investigators in other mosses, together with the work of Philibert and Milde, these results cannot be accepted as conclusive of the place and method of sex determination in this group; further research is necessary before a definite statement on this subject can be made.

The supposition that sex determination takes place at some fixed stage in the life-history of the plant, and that it is brought about by the separation of chromosomes, obviously leads to many difficulties. It is therefore suggested that sex is determined by certain metabolic processes

which are spread over a considerable number of cell generations, and which, as a general rule, are unaffected by external conditions. It is possible that these processes are initiated at a certain stage in the life-history, but it is unlikely that they depend on the separation of actual protoplasmic masses at any particular cell-division. If this view is accepted, variations from the normal sexual condition of a species may be explained by assuming the presence of some unusual factor which has interrupted the normal course of metabolism. No adequate explanation of such sexual abnormalities has been given by the upholders of the theory of sexual determination by the separation of determinants at some particular division. If, however, the view outlined above is accepted, the explanation of such cases becomes very considerably simplified.

In conclusion, it may be pointed out that the majority of investigations on the subject of sex determination have been carried out on animals. The conclusions so arrived at cannot be justly extended to plants, which differ fundamentally from animals in the possession of a definite alternation of generations in their life-history. The fact that in animals meiosis always corresponds with the gametogenic divisions, while this is rarely or perhaps never the case in plants, renders comparison of the two groups very difficult.

Similar investigations on plants have almost all been confined to Angiosperms, in which the alternation does not result in sharply distinguished generations. It is probable that further research of a similar nature carried out on the Bryophyta and Pteridophyta, in which the generations are always distinct and, in the latter group, usually lead an independent existence, would give valuable results.

#### SUMMARY.

1. An axis of *Mnium hornum* is described, bearing normal antheridia, bisexual organs, and modified archegonia.
2. The spermatogenic cells of the normal antheridia possess six chromosomes and, since this is the normal gametophytic number, the plant in question cannot have been produced aposporously.
3. The results obtained by É. l. and É. m. Marchal are discussed, and it is suggested that sex determination is not bound up with meiosis, but is brought about by metabolic processes which operate in the organism over a considerable part of its life-history.

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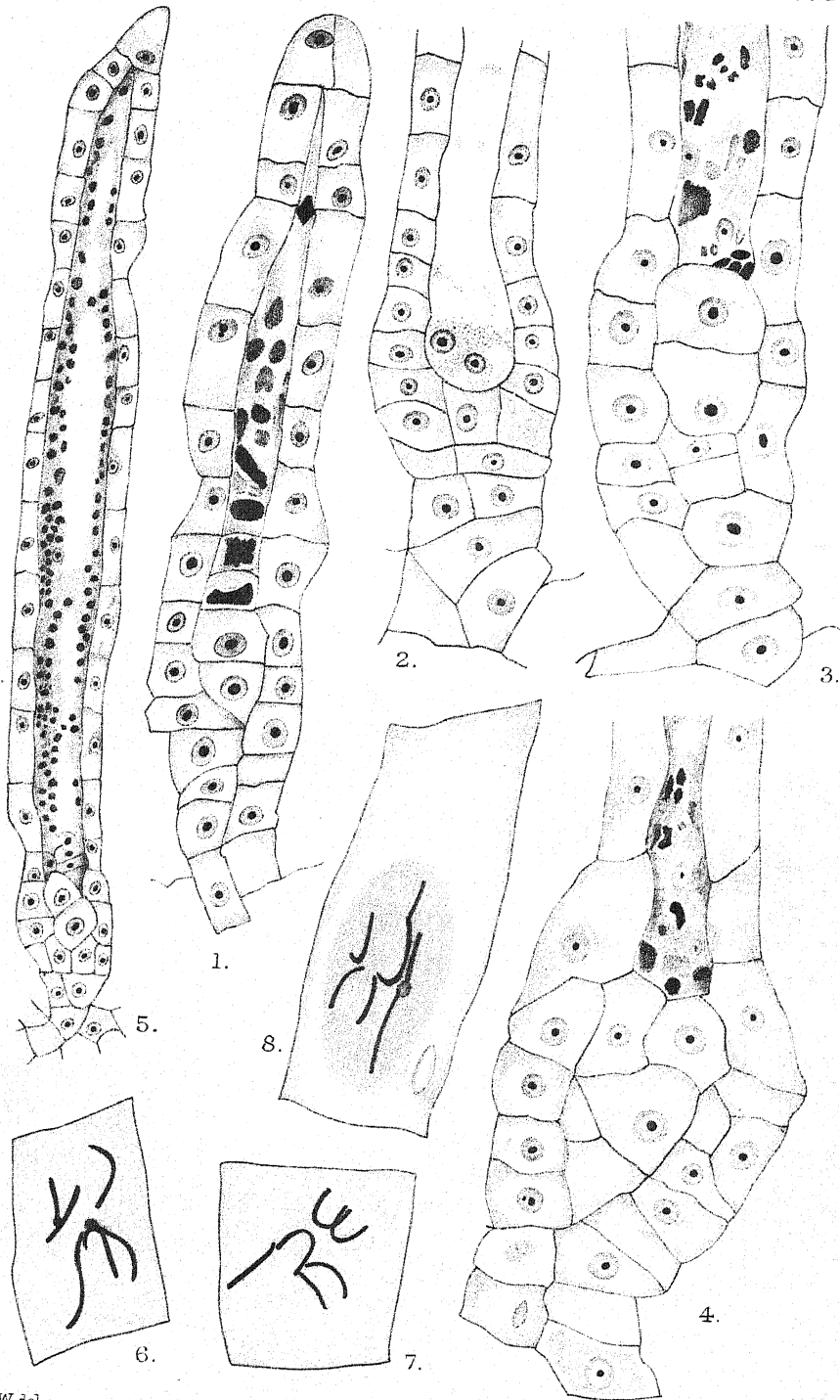
## EXPLANATION OF PLATE XX.

Illustrating Dr. Wilson's paper on Sex Determination in *Mnium hornum*.

All the figures were drawn with the camera lucida, Figs. 1-4 under a 2 mm. apochr. hom. imm. Zeiss N. A. 1.40 with comp. oc. 4,  $\times 500$ ; Fig. 5 under a D achrom. Zeiss with comp. oc. 4,  $\times 220$ ; Figs. 6, 7, and 8 under a 2 mm. apochr. hom. imm. Zeiss N. A. 1.40 with comp. oc. 18,  $\times 2250$ .

All the figures refer to *Mnium hornum*.

- Fig. 1. A young modified archegonium, showing division of the neck canal-cells.  
 Fig. 2. A mature modified archegonium in which the contents of the neck have been ejected; the naked cells in the venter are probably the ovum and ventral canal-cell.  
 Fig. 3. A modified archegonium, showing spermatogenic cells (?) in the neck-canal.  
 Fig. 4. A modified archegonium, showing spermatogenic cells (?) in the neck-canal.  
 Fig. 5. A bisexual organ, showing spermatids in the upper part and an ovum (?) and ventral canal-cell (?) in the lower part.  
 Figs. 6-8. Spermatogenic cells from a normal antheridium in late prophase of division, each showing six chromosomes. A chloroplast is seen in the right-hand lower corner of the cell shown in Fig. 8.



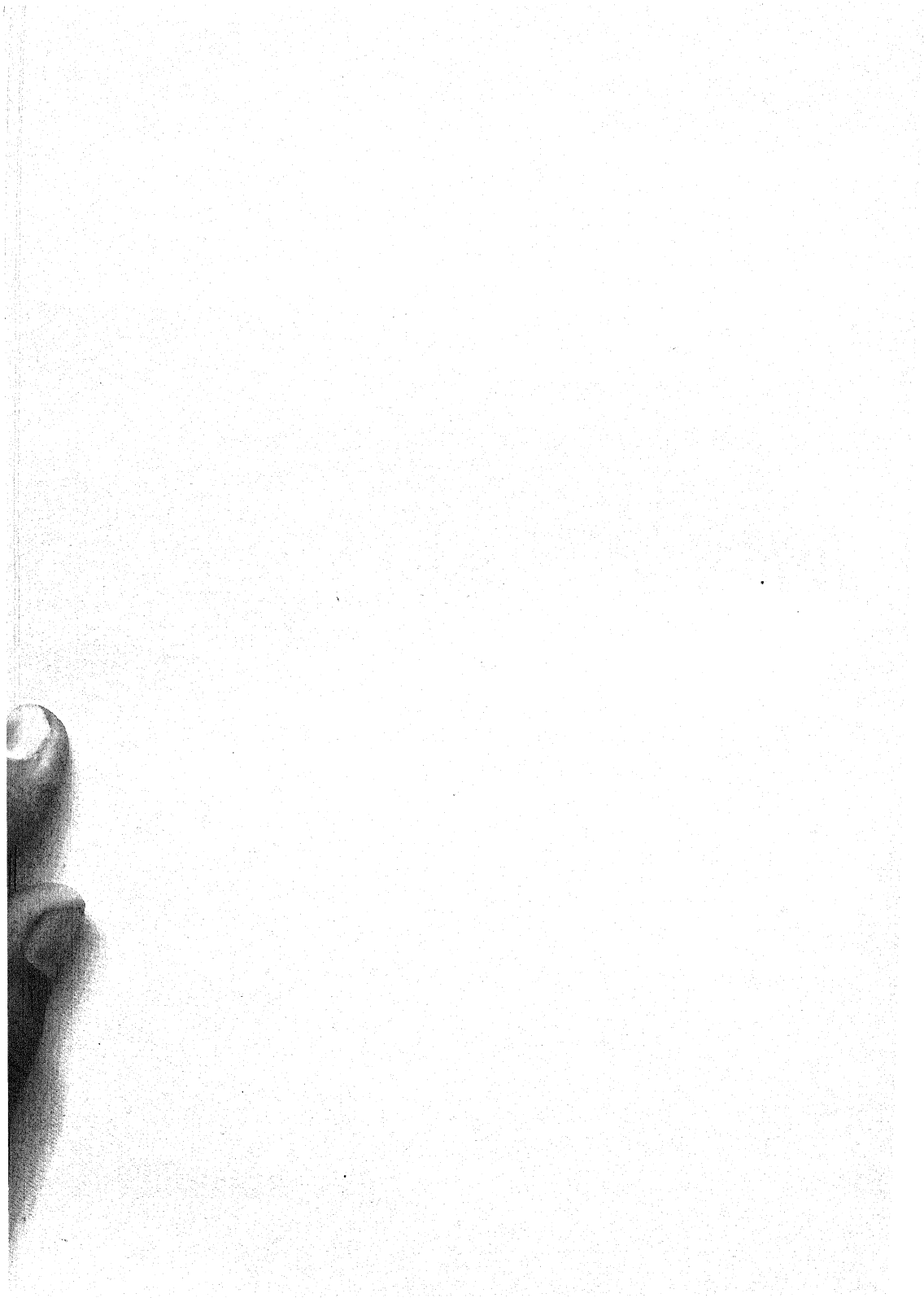
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# Spermatogenesis in *Mnium affine*, var. *ciliaris* (Grev.), C.M.

BY

WILLIAM L. WOODBURN,

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With Plate XXI.

CONSIDERABLE interest has recently been manifested in cytological investigation of antheridial tissues and sperm-cells of the Bryophyta. Inasmuch as quite different results have been described by various writers, it has seemed to the author worth while to present here a few observations concerning *Mnium affine*, var. *ciliaris*. Since Wilson (6), Allen (1), and others have quite thoroughly reviewed the literature upon the subject, it seems unwise to incorporate here that which would be largely a repetition of their work. Only a few points concerning which somewhat different observations have been reported may be mentioned briefly.

In my paper (8) on 'Spermatogenesis in *Blasia pusilla*' reference was made to Wilson's work (6) on *Mnium*, *Atrichum*, and *Pellia*; also to Allen's (1) investigation of *Polytrichum*. Wilson describes for *Mnium* and *Atrichum* the nuclear origin in the androcyte of a unique structure which he terms a 'limosphere'. The latter is composed of rod-like structures developed from material passed out from the nucleolus. Two other divisions from the nucleolus occur, one forming an accessory body, probably similar to the 'Nebenkörper' described by Ikeno (3), the other functioning as the blepharoplast. In *Pellia* Wilson finds centrosomes and centrospheres present during the last division of the spermatogenous tissue. He is convinced that in this case the centrosome persists and functions as the blepharoplast. A limosphere and an accessory body are also present in the androcyte of *Pellia*.

Allen (1) has followed the cytological details of the spermatogenous tissue of *Polytrichum* up to the beginning of the transformation of the androcyte. Consequently he did not have occasion at that time to observe, if present, the structures referred to above in Wilson's paper. However, during the earlier as well as later divisions of the spermatogenous tissue, Allen finds certain interesting structures. Kinoplasmic material becomes differentiated as plates which occupy the broad poles of the spindles during



karyokinesis. These polar plates are frequently observed to consist of individual granules, the kinetosomes. These plates of kinetosomes seem to actively function in spindle formation. When the cell-wall is formed only one plate is left in each cell. This plate divides and the two daughter-plates take up positions respectively at the poles of the succeeding spindle. In the androcyte mother-cell, however, instead of a plate only one granule appears at the spindle pole. As the groups of kinetosomes in the earlier generations divide into two daughter-plates preceding spindle formation, so this single polar body divides into two daughter-bodies which occupy respectively the poles of the last androgonial division. Allen is convinced that each of these two polar or central bodies persists in its respective cell, or androcyte, and there functions as the blepharoplast. No observations were made which would settle definitely the origin of the central body in the androcyte mother-cell.

For convenience, part of the terminology suggested by Allen will be used in this paper. The word 'sperm', however, is used instead of antherozoid. Allen designates the cell which is to be transformed into the sperm as an 'androcyte', the cell generation just preceding the androcyte as composed of 'androcyte mother-cells', and any spermatogenous cell of a still earlier stage as an 'androgone'.

Walker (5) finds that in the earlier divisions of the spermatogenous tissue of *Polytrichum formosum* the spindle poles are blunt, while in the last division fibres radiate from a centrosome-like body. These bodies were first observed on opposite sides of the nucleus. Walker is convinced that these centrosome-like bodies persist in the androcytes and function as the blepharoplasts. During the transformation of the androcyte, chromatin masses are extruded from the nucleus into the cytoplasm. These masses of chromatin probably correspond to the 'chromatoiden Nebenkörper' described by Ikeno (3) for *Marchantia* and the 'limosphere' reported by Wilson (6) in *Mnium hornum* and *Atrichum undulatum*. As the sperm develops, an arched band, probably similar to the 'cytoplasmatischer Fortsatz' of Ikeno, connects the blepharoplast with the nucleus. The development of this band may be at the expense of the extruded chromatin bodies. This connecting band may later be absorbed by the nucleus.

Black (2) finds the blepharoplast in *Riccia Frostii* appearing first in one angle of the androcyte as a sharply differentiated part of the cytoplasm. A small granule may sometimes be observed occupying each pole of the last division spindle, but no evidence was obtained to show that these polar bodies or granules persisted to function as the blepharoplast. The latter develops along the periphery of the androcyte as a homogeneous appearing cord. Neither the 'cytoplasmatischer Fortsatz' nor the 'limosphere' and 'chromatoiden Nebenkörper' referred to above were observed by Black. As the cord-like blepharoplast elongates the nucleus assumes a

crescent shape, becomes homogeneous in appearance, and closely applied to the blepharoplast. The latter extends forward as a narrow thread terminating in a thickened head which bears two cilia.

The writer has spent some time in studying certain of these features in *Porella*, *Marchantia*, *Fegatella*, and *Blasia*. A statement made in regard to the development of the sperm of *Blasia* (8) seems to be quite as applicable to the three others just mentioned. It seems also to be true of *Riccia Frostii* as described by Black. 'The blepharoplast makes its appearance first as a dense area of cytoplasm in opposite ends, respectively, of each of the pair of spermatids. Gradually a definite granule or body is differentiated, which develops as a thread or cord around the cell near to the plasma membrane. This cord, the blepharoplast, stains homogeneously throughout. Following its course the nucleus lengthens in close contact with the blepharoplast, the two become indistinguishable by the time one complete turn is made, and the body of the sperm, which stains like chromatin, continues to increase in length until the mature form is reached. Two cilia are developed probably from the forward end.' The writer has not observed in these forms the 'cytoplasmatischer Fortsatz', the 'limosphere', or the 'chromatoiden Nebenkörper' referred to above. While polar bodies were observed occupying the respective poles of the last division in *Marchantia*, it was not found possible to trace them as bodies of morphological rank throughout the telophase of this division.

When we compare these results obtained from the Liverworts with those recently described for species of Moss by Wilson, Allen, and Walker, there seems to be a considerable difference in respect to the details of development. Recent investigation, however, has led me to believe that the difference is not so great as at first seems apparent. It might be well at this point to call attention, on the other hand, to certain phenomena which seem to be quite constant, judging from recent reports, throughout spermatogenesis in both groups of the Bryophyta. Obviously there is a general agreement as to the form of the mature sperm. This body consists of a crescent-shaped, curved, or coiled slender portion representing nuclear material, while projecting beyond one extremity, which may be termed the anterior end, is the blepharoplast bearing two very delicate slender cilia. Occasionally a vesicle is attached to the posterior portion of the sperm. The exact character of this vesicle remains as yet somewhat uncertain. Walker (5) considers the vesicle to be made up largely of extruded chromatin, Wilson (6) thinks that material extruded from the nucleus is present in the vesicle, while Black (2) and Woodburn (7 and 8) have referred to it as cytoplasmic in nature.

Practically all of the writers too agree quite closely in their descriptions and figures of the early stages of the androcyte. This cell consists of a well-defined and relatively large nucleus, a surrounding zone of cytoplasm

bounded apparently by a very delicate plasma membrane, and situated somewhere within the cytoplasm there appears quite early a conspicuous dark staining body, usually more or less spherical at first, the blepharoplast. The origin and nature of this body is as yet a matter of dispute. It evidently functions in the development of the cilia, and whether it has to do with the growth processes of the latter or with the change in form of the cell, it or some portion of it certainly forms the base of attachment for the cilia.

The two stages wherein there seems to be more general agreement, the androcyte and the mature sperm, may be considered momentarily resting or fixed conditions, that is, for a very short time at least, there seems to be no noticeable change of form or internal structure. This may account for the fact that these two stages appear to be most constant throughout the various genera of the Bryophyta. It is during the very active conditions of karyokinesis which lead directly to the formation of the androcytes, and during the transformation of the androcyte into the mature sperm when the internal cell structures are rapidly changing and are certainly in an exceedingly high state of plasticity, that decidedly varying observations have been recorded. These facts, coupled with the extremely small size of the cells, make it rather difficult to follow with certainty the minute details of development.

The writer has been engaged for some time past in studying spermatogenesis in certain of the Musci, especially in *Mnium affine*, var. *ciliaris*. While it seems quite probable that many things have been elusive, and doubtless there is much yet to be recorded, it seemed wise to present here a few observations.

The antheridial heads were killed in chrom-osmic-acetic acid according to the formula of Mottier (4), washed, dehydrated, and embedded in paraffin in the usual way. The sections were for the most part cut five microns thick. For staining purposes aniline-safranin and gentian-violet were used with good results, also Haidenhain's iron-alum-haematoxylin counterstained with Bismarck brown. The best material was secured and fixed in the field one afternoon, May 10, after a heavy rain with the temperature near 70° Fahrenheit. The tissues were well supplied with water, the plants were in good condition, and many dividing nuclei were found. While the Moss has developed considerable adaptation to drought, yet it is noticeable that the tissues respond very quickly to changes in moisture.

Certain slides were also prepared of sperms which were allowed to escape in a drop of water, and while in the free swimming state killed with 2 per cent. osmic acid. These were stained with aniline-safranin and gentian-violet.

## RESTING STAGES AND PROPHASE IN THE ANTHERIDIAL TISSUES.

It may be well to speak briefly of the condition of the earlier cell generations in the antheridium. The cells are somewhat larger in the young antheridia than those found in the mature condition. Compare Pl. XXI, Fig. 1 with Figs. 8, 9, and 10. Much growth, however, takes place as the cells of the later generations have not diminished proportionately in size, in comparison to the number of divisions which have occurred, neither do the cell-contents seem to grow depleted. (Cf. Fig. 1 with Fig. 10.) The cytoplasm is finely granular, sometimes showing a tendency to form more or less flocculent masses (Fig. 1). Again, it may be observed very evenly distributed throughout the cell without any noticeable lumps or flakes (Figs. 3, 4, 5, and 6). The appearance of the antheridium from which the latter figures were drawn suggested most excellent fixation; however, it may also be true that the flocculent nature of the cytoplasm shown in Fig. 1 may be a natural condition and not an artifact. I have observed that in the deeper tissues, in those cells nearer the base of the antheridium where the fixing fluid does not penetrate so quickly and with as great strength as it does nearer the tip, frequently, although not constantly, there is a tendency towards vacuolization. The clear central part of the nucleus around the nucleolus is larger, with the chromatin network collected nearer the nuclear membrane, while the cytoplasm tends to collect in lumpy masses nearer the plasma membrane. A careful study was made with these facts in mind in an endeavour to give here representative figures.

A nuclear membrane is clearly present during the resting stage. The contents of the nucleus consist of a very conspicuous deeply staining and relatively large nucleolus surrounded by a clear region, which separates the nucleolus from a peripheral chromatin network (Figs. 1, 2, and 3). Walker reports practically the same condition in the resting cells of *Polytrichum*, but also describes and figures delicate threads connecting the nucleolus with this reticulum. The writer was not able to observe these threads in *Mnium*. The chromatin network is often partly obscured by a very finely granular substance, which appears usually during active conditions of prophase. This may be material which at other times, either earlier or later, may be represented in the substance of the nucleolus or chromatin and is at this particular stage in a transitional condition. At least it is quite clear that as the nucleus prepares to divide the nuclear network becomes more prominent, the lumps tending to draw out along the connecting threads (Fig. 3), forming eventually a distinct spireme (Fig. 4). During this process the nucleolus gradually loses its affinity for the stains (Figs. 1, 2, and 3) and finally is lost sight of altogether (Fig. 4). It is interesting to note that as the nucleolus apparently loses material the chromatin is most densely aggregated

immediately upon the periphery of the clear surrounding area (Figs. 2 and 3). This suggests a supply of material passing from nucleolus to chromatin in a plastic condition, not as already formed chromatin granules.

#### THE FORMATION OF THE CHROMOSOMES.

The condition represented in Fig. 3 passes very quickly over into the spireme stage (Fig. 4). By this time the nuclear membrane and also the nucleolus have disappeared, and the spireme seems to be loosely coiled throughout the nuclear region (Fig. 4). The spireme segments transversely into six chromosomes (Fig. 4*a*). Numerous counts were made, and while the chromosomes are so small and slender and somewhat intertwined in this stage, six seems to be the correct number. Six chromosomes were also counted in *Polytrichum commune* (Figs. 32 and 33). Fig. 31 shows the condition of the chromatin in *Polytrichum commune* just prior to the stage represented in Fig. 32. A spireme is apparently formed, but is much more closely wound than in *Mnium* (cf. Figs. 4 and 4*a* with Figs. 31 and 32). The chromosomes in *Mnium* are long and slender as they pass to the poles (Fig. 4*b*), where they unite again into a spireme condition (Fig. 5).

#### TELOPHASES.

Fig. 5 shows one cell in an early telophase which, judging from the size of the antheridium and the fact that the cells are beginning to separate and round off from each other, seems to be the last division of the spermatogenous tissue. No division that can be termed diagonal occurs in *Mnium affine*, var. *ciliaris*. The androcytes in their earliest condition are quite regularly found lying singly. Occasionally there may be the slightest suggestion of a pair. Whereas in the majority of the Liverworts it is quite easy to determine, as early as the time of metakinesis, by the position of the spindle, whether one is dealing with the last division or an earlier one, in the case of *Mnium* it is much more difficult. My observations lead me to believe that the androcytes round off from each other and enter upon the succeeding changes quite slowly and gradually until certain stages are reached, when, probably due to favourable environmental conditions, the remaining phases of transformation are passed through quite rapidly. The discussion of the further development of the androcyte will be taken up later. A study of the stages represented by Figs. 5 and 6 shows that practically the same processes in reverse order occur during the reorganization of the nuclei as occurred during the prophases. In Fig. 5 each daughter-nucleus has passed over into the spireme stage, while in Fig. 6 the spireme is giving way to a chromatin network surrounding a nucleolus (cf. Fig. 6 with Figs. 1, 2, and 3). The first indications of a cell-plate are shown by the thickening of the spindle fibres near the middle (Fig. 5), while in Fig. 6 a delicate cell-plate is shown extending almost completely

across the cell. By this time the daughter-nuclei have returned to approximately the condition of prophase shown in Figs. 2 and 3. During these stages as represented in Figs. 4, 5, and 6; no lumps, cytoplasmic plates, or polar bodies of any sort could be detected in the cytoplasm, and the cells seemed to be perfectly fixed and were very carefully stained.

#### THE ANDROCYTE AND ITS DEVELOPMENT INTO THE MATURE SPERM.

The first evidence that the last division of the spermatogenous tissue has occurred is the rounding off of the cells (Figs. 7, 8, and 9) and the appearance near the periphery of the cell of a densely staining body, the blepharoplast (Fig. 10). As was stated above, the last division of the spermatogenous tissue is not diagonal, so that frequently antheridia are found crowded full of such cells as are represented by Figs. 7, 8, and 9, but quite often more angular in outline than these figures. The cells apparently do not noticeably round off or separate from each other until this last division is complete. The tissue from which Figs. 3, 4, 5, and 6 were drawn was evidently undergoing this division as the cells were beginning to separate and round off slightly. Only in cells which have become free from each other have I found the development of the dark body, the blepharoplast, shown in Fig. 10. In the earlier cell generations of the antheridium no such body was discovered. The evidence obtained here in regard to this body points to an origin similar to that which the author has previously suggested for *Blasia*, *Porella*, and other Liverworts. In *Blasia* (8) the first indication of the blepharoplast is a dense area of cytoplasm. A definite granule is differentiated apparently in this dense area and develops as a homogeneously staining thread or cord around the cell near the plasma membrane. A similar process obtains in *Mnium* (Figs. 11 and 12). The nucleus takes up a position to one side of the cell, so that as the blepharoplast grows in length the latter comes in close contact with the nucleus. For some time the blepharoplast may be observed as distinct from the nucleus; then the two seem to become more or less fused together. It is during this stage of development that the most varying accounts have been given. It seems to me not surprising that such has been the case. At a time like this, when protoplasmic structures are changing so rapidly, slight differences in the methods of handling the material or differences in growth conditions at the time of fixation may very greatly alter the appearance of certain parts. Walker (5) has called attention to the extreme sensitiveness of such cells as these to changes in the environment. As I have already suggested, these facts should at least be kept in mind by any one investigating this stage of development.

Although Wilson (6) and Walker (5) agree that, as the nucleus takes up its final position and changes to the form found in the mature sperm, the

chromatin is not all retained within the nuclear membrane, but that at least a considerable portion is extruded into the cytoplasm, no agreement has yet been reached as to the exact fate of this nuclear material after passing out into the cytoplasm. The problem seems to be more difficult than that met with in the Hepaticae, where the nucleus, in the majority of cases observed, remains entire and lengthens out bodily in the direction taken by the blepharoplast. Wilson (6) has, however, cited *Pellia epiphylla* as showing much the same condition as he found in *Mnium hornum* and *Atrichum undulatum* in the Musci. My observations on *Mnium affine*, var. *ciliaris*, lead me to believe that during this stage the nucleus does not remain as sharply differentiated as in the case of the Liverworts, but that there is a tendency for the nuclear membrane to disappear, allowing possibly a more intimate association between nuclear and cytoplasmic material (Figs. 13 and 14). The nuclear content becomes very finely granular, staining almost homogeneously (Figs. 12 and 14). In Fig. 12 are shown two lumps which may represent nucleoli; the remainder of the nucleus stains homogeneously. As development proceeds, the nucleus becomes even more smoothly and evenly granular (Figs. 12 and 14), these lumps or nucleoli as well as distinct chromatin granules disappear, the nucleus stretches out in a direction parallel with the blepharoplast and at the same time loses to a certain extent its very distinct outline. Occasionally very dark staining lumps may be observed in the cytoplasm, and the nuclear part may also be somewhat vacuolate (Fig. 13). The nuclear membrane, if present, stains very faintly and can be scarcely differentiated in Figs. 13 and 14. Figs. 10, 11, 12, 13, 14, and 15 represent quite closely consecutive stages, so that the position of the nucleus in each case can be readily determined. Fig. 15 represents a well-advanced stage in which the nucleus and doubtless also the blepharoplast have formed one complete turn, while the latter may be readily distinguished from the former. Fig. 21 represents a stage not greatly in advance of that shown in Fig. 15. The cause of the difference in appearance may be due either to the fact that Fig. 15 represents a sperm killed and fixed inside of the antheridium, while the one shown in Fig. 21 had been allowed to escape in a drop of water on the slide and then very suddenly killed with 2 per cent. osmic acid, or the former may probably represent a sperm normally smaller. Either one or both explanations together are quite plausible. Cilia could easily be seen in Fig. 21, but they could not be detected in Fig. 15. Figs. 10, 11, 12, 13, 14, 15, and 16, while presenting certain features which will be discussed later, demonstrate clearly two or three prominent things. The blepharoplast grows as a cord, or band around one side of the cell. The nucleus moves to this side of the cell and lengthens in a course parallel with that taken by the blepharoplast. While these changes in form are taking place, the internal structure of the nucleus changes from a coarse open network to a very smooth, granular, homo-

geneously staining substance. Vacuoles and dark staining lumps may appear in the cytoplasm (Figs. 13, 14, 16, 20, 22, and 23). The part apparently representing the nucleus may also appear vacuolate. A very close association seems to exist between nucleus and cytoplasm on the one hand, and nucleus and blepharoplast on the other. Probably there is a free interchange of materials at certain stages between these three structures. There does not seem to be the appearance, with sufficient constancy, of structures other than the nucleus, the cytoplasm, and the blepharoplast with the cilia, to warrant a separate designation of other bodies such as limosphere or accessory body. Furthermore, as development proceeds there is the tendency for the sharp distinction between nucleus, cytoplasm, and blepharoplast to disappear.

#### VACUOLES IN THE CYTOPLASM OF THE SPERM-CELL.

The structure described by Wilson as a limosphere is a spherical body which may contain substance staining like cytoplasm, or may be hollow in the nature of a vacuole. Certain of Wilson's figures of the 'limosphere' correspond quite closely to my Figs. 14, 16, 23, and 28. Black also reports a vacuole in the cytoplasm of *Riccia Frostii*. Figs. 22 and 23 represent nearly the same stage of development, but do not represent similar conditions of vacuolate cytoplasm. The cytoplasm in Fig. 22 contains a number of vacuoles, while that in Fig. 23 contains only one. Such a vacuole as is shown in Fig. 23 gradually disappears (Figs. 28 and 29). The relation as regards position which this vacuole bears to the main body of the sperm suggests that the latter may receive the finely granular substance which bounds the former. The small granule shown in Fig. 14 is not in the centre, but lies at least near and probably upon the surface of the vacuole. The granule in Fig. 15 may represent the same kind of body. Possibly these are nucleoli which have become free in the cytoplasm. Nucleoli, or fragments of nucleoli, are doubtless frequently left free in the cytoplasm of higher plants during karyokinesis, when the nuclear membrane does not longer separate chromatin and cytoplasm. There is, at least, considerable evidence to support the theory that during this transformation of the sperm-cell, the nuclear membrane may to a certain extent break down (Figs. 13, 14, and 16), and that an exchange of substances between nucleus and cytoplasm may take place.

A slightly different condition is represented in Figs. 21, 25, 26, and 27, where we do not find the spherical vacuole, but a region or group of vacuoles. The granular substance connected with these, instead of forming a hollow sphere, produces a distinct network or mesh, which reminds one very much of the vesicle that has been described for the sperm of more or less closely-related forms. That this vesicle also gradually disappears is clearly evident from a comparison of Figs. 21, 25, 26, and 27. The fate



of this vesicular structure is certainly the same as that of the vacuole just described in the preceding paragraph. In fact, I can see no reason for making a distinction between the two, except that the former (Figs. 16, 20, 23, and 28) seems to represent a single large vacuole, while Figs. 21, 25, 26, and 27 seem to represent a cluster of smaller ones. One characteristic of the latter should be mentioned which does not appear in the figures. There is a very decided contrast between the finely granular substance of the nuclear portion and the strands of this vesicle, which was very difficult to represent in the drawings. While these vesicular strands are very distinct, they do not stain like chromatin, and they have a lighter bluish appearance when compared with the darker richly staining substance of the part, which certainly contains chromatin.

#### THE DEVELOPMENT AND NATURE OF THE BLEPHAROPLAST.

While the general course of the development of this structure has been described, there are certain features which deserve special mention. Under certain conditions, doubtless of staining and fixation, possibly however due to different conditions of growth at the time of fixation, the distinction between the band or cord, which we have called the blepharoplast, and the nucleus can be traced to a much more advanced stage than is possible at other times. For instance, compare Fig. 16 with Fig. 21. In the former, although the body of the sperm has made slightly more than one complete turn, the blepharoplast cannot be distinguished, while in Fig. 21, which seems more nearly mature, a distinct cord is visible throughout the entire length. From Fig. 22 throughout the remaining stages represented, no cord distinct from the other material is evident.

A majority of the figures of sperms have been drawn from what may be termed the side-view. Many sections were found presenting the sperm-cells to the observer from almost every conceivable angle. A number of these views are shown in Figs. 17, 18, 19, and 20. Observations of this sort bring out certain facts which are not otherwise evident. Fig. 17, which is drawn from a view-point on a radius passing through the blepharoplast, shows the latter structure to be a relatively broad band passing between the observer and the nucleus. Fig. 18, which is more distinctly a surface view drawn from the same angle as Fig. 17 and representing a somewhat more advanced stage, shows a band considerably broader in proportion than that in Fig. 17 and with much denser contents along one side than the other. The nucleus is not shown in this figure. Figs. 19 and 20 represent cross-sections through stages such as are shown by Figs. 16 and 23. Both Figs. 19 and 20 had the stain quite well washed out, and they were also drawn rather lightly. The nucleus has apparently all collected along the blepharoplast, and the two are in cross-section indistinguishable.

Quite an open network of cytoplasm is still present. Cross-sections of the blepharoplastic and nuclear band are here obtained. In Figs. 17 and 18 the cross-section of this band forms a rather flat ellipse quite dense in contents throughout. Fig. 19 represents a very thin section through a cell where the nucleus is certainly well distributed into or along the blepharoplast, the two forming a common cord or band. As the section passes through this band four times it has evidently completed more than one full turn and a half. Three sections of the band are elliptical in outline and densely granular; the fourth section is more circular in outline and hollow, indicating that at this point, which may have been near the middle, the structure in question was tubular. The two sections of the band which are shown in Fig. 20 are both nearly circular and more or less hollow, indicating here also a tubular structure. The vacuole noted elsewhere is shown to lie, sometimes at least, to one side of the nuclear band (Fig. 20). The fact that the body of the sperm as first formed is often tubular or vacuolate through the centre, helps no doubt to explain the process whereby this structure lengthens and grows less in diameter. A section passing in a somewhat obliquely horizontal plane through such a cell as is shown in Fig. 16 might very readily make three cross-sections of the nuclear band, and the appearance of this figure in side-view would suggest the probability that one section would be vacuolate in the centre and the other two more nearly homogeneously granular throughout. (Compare such an imaginary cross-section with Fig. 19.)

Summing up briefly conclusions in regard to the nature and development of the blepharoplast, as suggested by an observation of Figs. 10-23, we find that a darkly staining granule or body, apparently a cytoplasmic differentiation, develops into a more or less radially flattened band along the edge of the cell in contact with the plasma membrane. This band, which we will call the blepharoplast, is at first situated independently of the nucleus, but the two soon come into close contact and finally seem to be fused into one homogeneously staining band. The original blepharoplastic band remains more densely staining in capacity for some time, though of apparently the same staining affinities (Fig. 21). This band, consisting of nucleus and blepharoplast, now forms the main body of the sperm, and cross-sections show that it is more or less radially flattened, at least towards the two ends, and in earlier stages a vacuole passes for some distance through the centre, making the structure more or less tubular, at least throughout part of its length. There is a very close association between the nucleus and blepharoplast, with probably some question as to whether these two bodies remain as distinct parts or become, to a certain extent at least, a homogeneous structure.

## FINAL STAGES IN THE DEVELOPMENT OF THE SPERM.

It has been suggested in the previous paragraph that two structures, the blepharoplast and nucleus, unite in producing the band- or ribbon-like structure of the mature sperm, which becomes coiled in the cell cavity. The cytoplasmic area within the coiled portion may contain one or more vacuoles (Figs. 22, 23, and 28) or a vacuolate area (Figs. 21, 25, 26, and 27); occasionally the cell may be free from vacuoles (Fig. 24). Surrounding the vacuoles material occurs which stains sometimes very much like the body of the sperm (Figs. 23 and 26) or more like cytoplasm (Figs. 21, 25, and 27). In Fig. 27 around one vacuole there is located densely staining material, while the remainder of the vacuoles are surrounded by strands or layers of material staining faintly, much like cytoplasm. Probably chromatin may, in greater or less quantities, be at times diffused into the cytoplasmic region. If such is the case, there does not appear to be any regular or constant structures formed from this diffused nuclear material. The blepharoplast is the only regular and constant formation in the cytoplasm. A study of the figures from Fig. 21 to Fig. 29 suggests very strongly that this material localized within the area surrounded by the coiled band is either consumed by, or incorporated within, the main body of the sperm. Fig. 30 represents the most mature condition observed, in which the sperm is beginning to stretch out and all indications of the formerly included vesicle have disappeared. The last traces of this vesicle may be observed on the inner surface of the sperm in Fig. 29, where a considerable portion does not present a smooth outline, but a surface roughened with granular material certainly remains of the material included in the vesicle of the preceding figures.

While it was impossible, as already stated, to differentiate in the last stages of development between blepharoplast and nucleus as two distinct structures, as can be easily done in the Liverworts, yet a study of Figs. 13, 14, 15, 21, and 24-30 suggests that they do remain distinct at least for a considerable length of time. It seems probable that the nuclear material is distributed, in some cases at least, along the entire length of the blepharoplast. It may be also that there is a more intimate association between the blepharoplast and nucleus in the Musci than in the Hepaticae. In Fig. 19 the blepharoplast is clearly seen projecting in both directions beyond the nucleus; in Fig. 15 it is extended as a sharply differentiated cord a distance beyond the posterior end of the nucleus. In Fig. 24 the blepharoplast with cilia attached projects beyond the anterior end of the nucleus, while in Figs. 25-30 one or both extremities of the sperm body are seen to be drawn out into slender, almost filiform, tips.

Figs. 25-30 indicate the difficulty in determining the origin of the cilia and their attachment in the earlier stages of development. This

is due to the fact that the cilia are extremely delicate and, until the sperm becomes free, are usually wrapped very closely around and parallel with the main body. Figs. 28, 29, and 30, however, show very clearly that the cilia are attached at the extremity of the anterior end of the sperm. I use the term 'anterior' to refer to that extremity of the sperm corresponding to the portion or region in which the blepharoplast begins to develop. As shown in Fig. 30, the mature sperm of *Mnium affine*, var. *ciliaris*, seems to be essentially the same as that described for other Liverworts and Mosses. The vesicle which has frequently been described is readily found in slightly earlier or younger stages (Figs. 27 and 28).

#### SUMMARY.

Resting stages of the spermatogenous tissue of *Mnium affine* var. *ciliaris* show the usual disposition of chromatin and cytoplasm. There is a very prominent, densely staining nucleolus, separated from a surrounding chromatin network by a clear area. The cytoplasm may be evenly and smoothly granular or slightly flocculent.

As the nucleus enters the prophase of division, the nucleolus stains more faintly, while immediately outside of the surrounding clear region the chromatin aggregates more densely. A coarse reticulum is formed which passes over into a clearly defined spireme. From the latter six chromosomes are differentiated.

So far as observed, the nuclear division proceeds in the usual manner without the accompaniment of polar bodies or plates.

The cell-plate seems to be formed in the usual way through cytoplasmic activity in the equatorial region of the spindle, and the daughter-nuclei are reorganized by passing through stages corresponding to those of the prophase, but in the reverse order.

No diagonal division was found to occur in either *Mnium* or *Polypodium*. This makes it rather difficult to identify the last division of the spermatogenous tissue until it is completed.

The first indications that this division is completed and that the androcytes have been formed is found in the separation and rounding off from each other of the cells. Next, the blepharoplast appears in the cytoplasm apparently as a cytoplasmic differentiation in the androcyte in which it functions.

The blepharoplast develops as a more or less radially flattened band in a course closely applied to the plasma membrane. The nucleus becomes closely applied to the blepharoplast, loses its coarse network and stains homogeneously, and lengthens parallel with and very closely applied to the blepharoplast. The development of the blepharoplast precedes that of the nucleus. The nucleus and cytoplasm during this process may not

be kept entirely separate, but there are indications of a certain amount of diffusion from one to the other.

As the blepharoplast and nucleus lengthen to form the mature sperm, they fuse more closely and eventually become indistinguishable, forming a homogeneous band or cord which in cross-section may be elliptical and densely granular or more nearly circular and hollow, showing the structure to be, at certain stages of its development, tubular throughout part of its length.

A vesicle, enclosed within the coiled body of the sperm and containing cytoplasm and probably some nuclear material, disappears as the sperm approaches maturity. The granular substance of the vesicle is apparently used up in the process of development, possibly being directly absorbed through the inner surface of the main portion of the sperm.

The mature sperm is long and slender, almost filiform, pointed at both ends, with two cilia attached at the forward extremity of the blepharoplast.

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## EXPLANATION OF FIGURES IN PLATE XXI.

Illustrating Mr. Woodburn's paper on Spermatogenesis in *Mnium affine*, var. *ciliaris* (Grev.), C.M.

The figures were drawn at table level with the aid of a camera lucida, a Spencer apochromatic immersion lens 1.5 mm. N.A. 1.30, and compensating ocular No. 12, giving a magnification of approximately 3,200 diameters.

Fig. 1. Androgone or cell from the spermatogenous tissue of a young antheridium, in a resting stage.

Fig. 2. Nucleus, from an older antheridium, entering upon the prophase of division.

Fig. 3. Cell from the same antheridium as Fig. 2, showing the collection of the chromatin into large lumps preparatory to spireme formation, and the nucleolus growing less in staining capacity.

Fig. 4. Spireme in a cell of the same group as Figs. 2 and 3. Probably just previous to the last antheridial division.

Fig. 4a. Spireme segmented into six chromosomes, which seems to be the gametophytic number for *Mnium affine*, var. *ciliaris*.

Fig. 4b. Late anaphase.

Fig. 5. Telophase of division immediately following Fig. 4.

Fig. 6. Later telophase and formation of cell-plate.

Fig. 7. Early stage of androcyte. Cytoplasm somewhat flocculent and slightly lumpy.

Fig. 8. Practically the same stage as Fig. 7, but showing more distinctly the chromatin network and the nucleolus.

Fig. 9. Also early stage of androcyte, showing nucleolus and the chromatin scarcely out of the spireme stage. About the same stage as Figs. 7 and 8.

Fig. 10. Androcyte with nucleus showing a nucleolus and chromatin network. A globular dark staining body, the blepharoplast, is present in the cytoplasm and the nucleus has shifted to one side of the cell.

Fig. 11. Androcyte with the nucleus containing a delicate chromatin network. The blepharoplast is considerably elongated.

Fig. 12. A little more advanced than Fig. 11. The nuclear content is becoming homogeneous, and the blepharoplast is growing in length.

Fig. 13. Blepharoplast extending at least half-way around the cell. The nucleus has become quite crescent-shaped, and a number of densely staining bodies are present in the cytoplasm.

Fig. 14. Blepharoplast still elongating and the nucleus being distributed along its course. A vacuole is present in the cytoplasm.

Fig. 15. Somewhat more advanced than Fig. 14, the blepharoplast being distinguishable only at the posterior end of the sperm. Indications of a vacuole similar to the one in Fig. 14.

Fig. 16. Sperm of about the same stage of development as shown in Fig. 15, but indicating much more rapid development. A vacuole is present in the cytoplasm, but the nucleus and blepharoplast seem indistinguishable.

Fig. 17. Approximately the stage as shown in Fig. 12, but viewed from the back (Fig. 12 may be considered a side-view). The blepharoplast is seen to be a flat band.

Fig. 18. From the same point of view as Fig. 17, showing only the surface. The blepharoplast appears to be darker along one edge.

Fig. 19. Cross-section through such a cell as shown in Figs. 16, 21, 22, or 23. The coiled portion of the sperm, now consisting of nucleus and blepharoplast, is seen cut in cross-section four times.

Fig. 20. Coiled portion of the sperm cut in cross-section twice. The vacuole noted elsewhere is present.

Fig. 21. Sperm allowed to escape and killed with 2% osmic acid. A densely staining cord, evidently the blepharoplast, extends throughout the length of the sperm. The nucleus is well distributed along the blepharoplast. A vesicle is contained in the cytoplasmic region and cilia are present.

Fig. 22. Drawn from sperm killed while still in the antheridium. More homogeneous throughout than Fig. 21. The difference may be due to conditions of less rapid development at the time of fixation or to the different condition under which fixation took place.

Fig. 23. Approximately the same stage of development as in Fig. 22, but the majority of the cytoplasm collected around a single vacuole.

Fig. 24. Slightly more advanced condition than Fig. 23. The coiled body of the sperm staining homogeneously, the cytoplasm somewhat flocculent but quite evenly distributed, and cilia attached to a slender forward projection, doubtless the blepharoplast.

Figs. 25-30 represent sperms which were allowed to escape into a drop of water from the antheridium and were killed on the slide with 2 % osmic acid. Some were allowed to remain longer than others in the water before the osmic acid was applied.

Fig. 25. Cilia are present and a vesicle is quite prominent in the cytoplasm.

Fig. 26. Cilia are present and the material of the vesicle is apparently being incorporated as a part of the main coiled portion of the sperm.

Fig. 27. Cilia are present. The sperm is beginning to uncoil and the vesicle is disappearing.

Fig. 28. Almost the same condition as found in Fig. 27. A single rather large vacuole, which certainly corresponds to the vesicle shown in the preceding figures, seems to be losing its substance to the main body of the sperm.

Fig. 29. Probably somewhat further developed than Fig. 28. The vesicle has almost completely disappeared, only a few fragments remaining on the inner surface of the sperm. The point of attachment of the cilia is very distinctly shown.

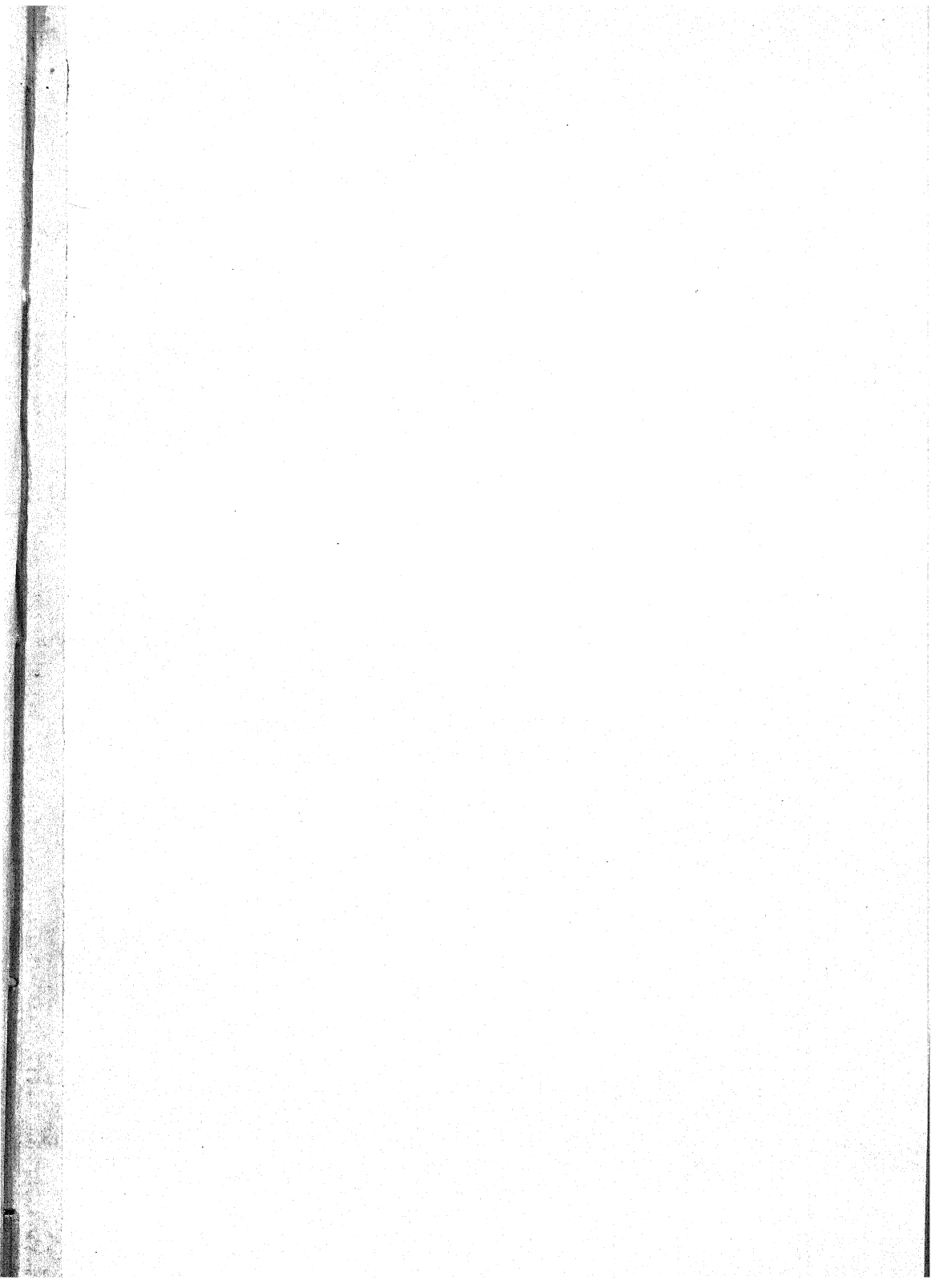
Fig. 30. Mature sperm. No indications of the vesicle remain. The sperm has evidently been free in the water for a considerable length of time.

Figs. 31, 32, and 33. *Polytrichum commune.*

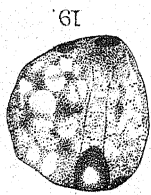
Fig. 31. Nucleus of an androgonia just previous to the differentiation of the chromosomes. Chromatin, probably in spireme condition, wrapped in a rather close knot.

Fig. 32. Differentiation of chromosomes. Six projections or loops are distinguishable.

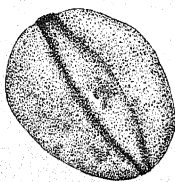
Fig. 33. Six chromosomes just previous to the metaphase of division.



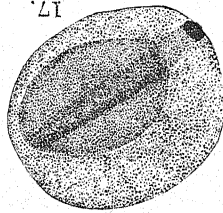




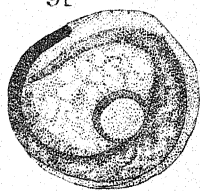
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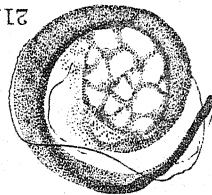
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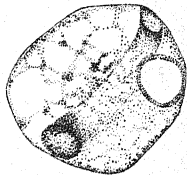
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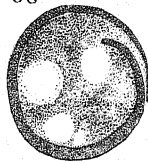
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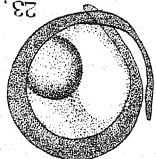
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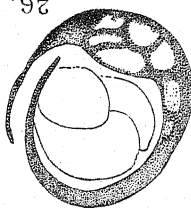
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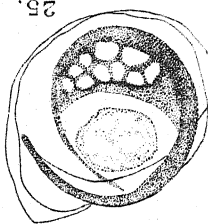
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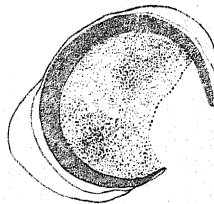
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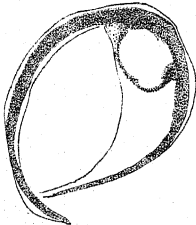
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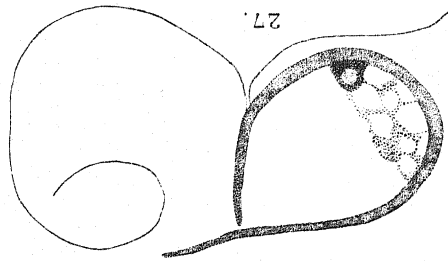
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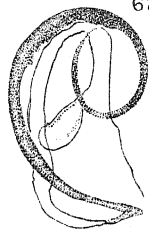
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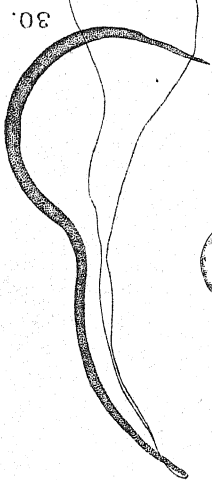
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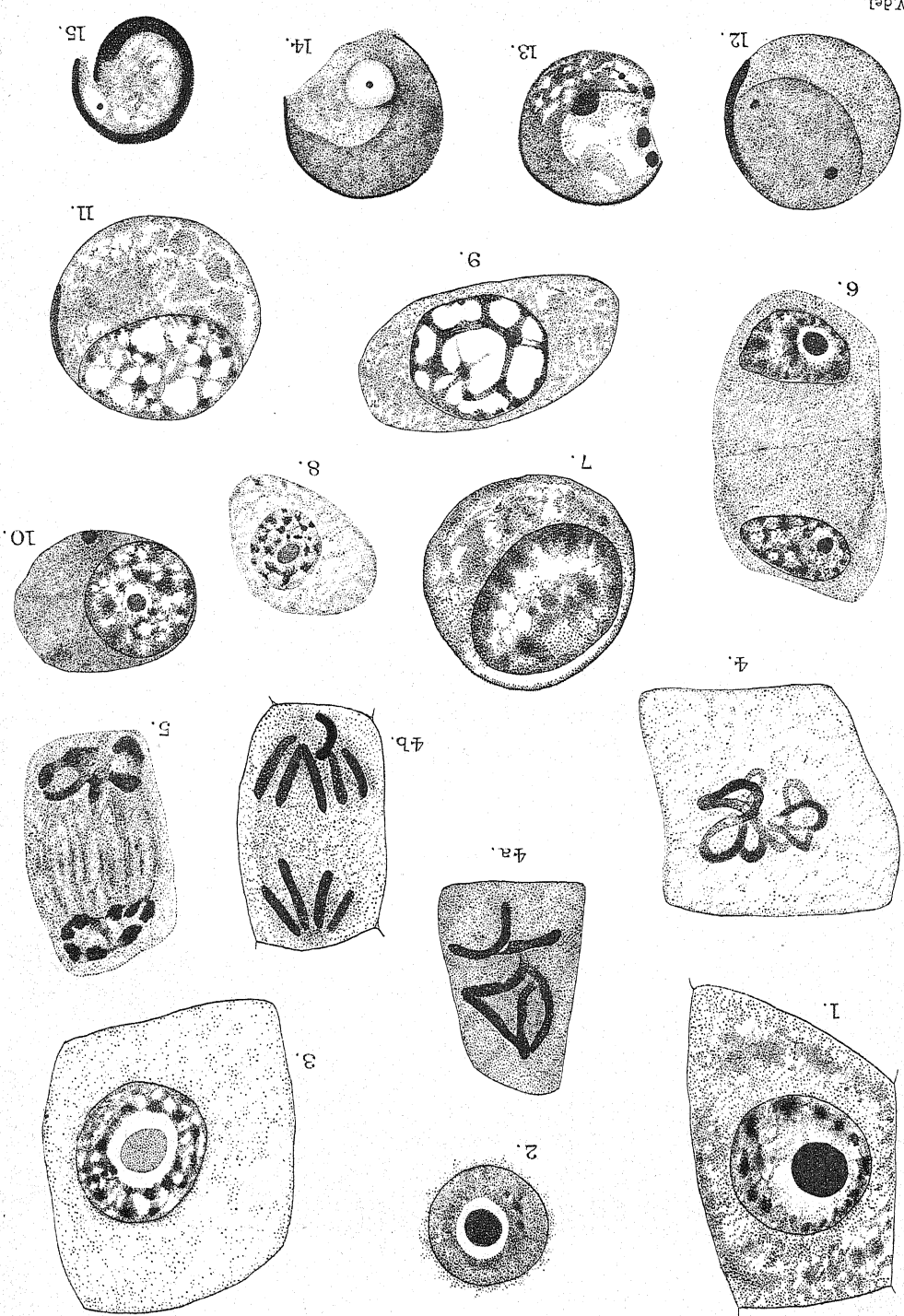
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# The Pollen-presentation Mechanism in the Compositae.

BY

JAMES SMALL, B.Sc. (Lond.), Ph.C.,

*Demonstrator in Botany, Armstrong College, Durham University.*

With seven Figures and two Tables in the Text.

## INTRODUCTION.

WHEN it is remembered that this order includes over 13,000 species, the very voluminous literature does not come as a surprise, but in spite of the numerous contributions to our knowledge of the Compositae, very few attempts have been made towards the elucidation of the evolution of even the main divisions of the order. Cassini (3), in 1826, made one of the first attempts to express the affinities of the tribe. He did so by placing the nineteen tribes, into which he divided the order, in an ellipse with the Vernoniées and Lactucées at one end and the Hélianthés at the other, with twelve lines crossing the enclosed space to indicate the presence of some common characteristic in tribes which he considered to be otherwise unrelated. In 1871 Delpino (5) gave a phylogenetic table for the Artemisiaceae showing the Compositae derived from the Campanulaceae through the Lobeliaceae and the Artemisiaceae derived from the Senecionidae, but since the Senecionidae, to which reference is made here, included many of the genera now in the Heliantheae, Helenieae, and Anthemideae, the value of this speculation, for it was little more, is seen to be negligible.

Bentham (1), in 1873, followed Cassini in his method of expressing the inter-relationships of the tribes with very little change, except in the reduction of the number of divisions to thirteen and the placing of the Senecionideae between the Anthemideae and Calendulaceae instead of next the Astereae, as Cassini had it. In considering the affinities, Cassini does not seem to have thought of one tribe or its ancestors having given rise to any other tribe, as is shown by the form of his diagram and the numerous cross-connexions introduced, but of course he thought in pre-Darwinian terms. Bentham took geographical distribution as his chief guide to the evolution of the different groups, and considered that the order could be detected at its earliest recognizable stage in Africa, Western America, and possibly

Australia. He regarded the Helianthoideae as nearest the primitive type, and evidenced the free anthers in the female flowers of a sub-tribe, Petrobieae, in addition to the presence of paleae on the receptacle and the paleaceous pappus of the tribe, in favour of this view. The capitula of the Petrobieae are, however, dioecious, and this sub-tribe of four species seems in several other characters to be far from primitive. From his remarks on the comparative antiquity of the tribes, one gathers that he considered there had been three main lines of development, the first giving the Eupatoriaceae, Vernoniaceae, Cynaroideae, and Mutiseae; the second giving the Helianthoideae, Helenioideae, Anthemideae, Asteroideae, Senecionideae, and Inuloideae; the third giving the Cichoriaceae.

There has been a number of efforts made to elucidate the phylogeny of the Cichorieae, mostly by French anatomists. Vuillemin (20), in 1884, after his elaborate research, concluded that anatomy was of very little use in the classification of this order. Col (4), in 1899, from his researches on the secretory canals and laticiferous tissue in the Compositae, concluded that the Liguliflorae were derived through the Arctotideae and Calenduleae from the 'Radiées'. Lavalie (11), in 1912, from his study of the development of the fruit, and in particular the structure of the mature pericarp, related the Liguliflorae to the Cynareae, on the one hand, through *Cichorium*, *Catananche*, and *Scolymus*, and to the Mutiseae through *Moscharia* on the other. Dufour (6), in 1907, and Lebard (12), in 1913, after studying the cotyledons of many seedling Cichorieae, applied their researches to the phylogeny of the genera in that tribe.

The classification of the family suffered many changes before the publication of the 'Genera Plantarum' (2), but since then Hoffmann (10), in the 'Pflanzenfamilien', has adopted Bentham's classification in all the chief points, and Wettstein (21) follows Hoffmann entirely. Small (19) raises the family to the rank of an order, which he names the *Carduales* and divides into three families—Ambrosiaceae, Carduaceae, and Cichoriaceae. The separation of the few abnormal genera included in the Ambrosiaceae seems rather unnecessary, and Hoffmann's classification is adopted in the following analysis of the variations in the characters of the essential floral organs, which forms the first of a series of studies undertaken with the purpose of elucidating the inter-relationships of the tribes.

#### POLLEN-PRESENTATION MECHANISM.

In order to arrive at a natural classification of this very homogeneous family, many attempts have been made to utilize characters which in more varied families are regarded as negligible. Even the achenial hairs have been studied for this purpose (15), but although the structure of the hairs of an order may prove valuable sometimes, as in the Cruciferae, in most cases

this is a very uncertain guide to relationship ; for example, the present writer (18) and also Schmidt (16) and Hoch (9) have studied the hairs of the families of the Tubiflorae, and it is found that, while some allied species or even genera may show very similar hairs in many cases, allied species may have hairs which are quite dissimilar. The study of the achenial hairs of Compositae leads to the same conclusion. Vuillemin (20) showed that anatomy was no certain guide to affinity, and later papers (8 and others) confirm his conclusions that anatomical characters are reliable only in certain special and very limited groups. The floral characters, therefore, become of supreme importance, and as they are so uniform throughout the order, the value of details in their structure must be considered carefully. Linnaeus naturally classified the group according to the condition of sexuality in the florets, but the first systematist to consider the matter in detail (i. e. Cassini (3)) saw at once that the dominating character was the pollen-presentation mechanism, and he accordingly gave the greatest value to the characters of the styles and stamens. Lessing (13) followed Cassini, but laid more stress upon the characters of the style in the delimitation of the larger groups, and in this he is followed by Bentham (2). For generic distinctions the venation of the corolla was used to some extent by Don, and the form of the achene by Schultz Bipontinus (17).

Although the value of the details of structure of the styles and stamens has thus been recognized for purposes of classification, the significance of these details to the plants themselves has been considered negligible. Of all recent synantherologists, Bentham gave the highest value to the variation in the appendages of the stamen, but he writes (1) : ' The anthers, however, are sometimes provided with certain appendages apparently of little or no functional or homological importance, but which, nevertheless, from the remarkable constancy of their presence or absence in whole tribes, supply one of the most valuable characters in Compositae if applied with proper caution.'

Since the highest development of these appendages is found in the same groups with the highest complexity in the structure of the style, it becomes highly probable that the appendages have quite a definite function, and a consideration of the facts shows that here, as in the rest of the characters, economy is the dominant factor. Economy of corolla material leads to the aggregation of the flowers into a capitulum ; economy of calyx material leads to the entire disappearance of that part of the flower or to its modification into a pappus ; economy of stamen material leads to reduction in number of the stamens to the minimum compatible with the efficiency of the pollen-presentation mechanism ; economy of carpel material leads to reduction in the number of carpels to two, and of ovules to one. From this it will be seen that the polliniferous tissue is the only

part of the flower in which the tendency to economy has not previously been recognized.

Considering the appendages of the styles and stamens in the light of the idea that economy of pollen is a factor in the success of the Compositae, the value of the hairs on the exterior of the style branches and the appendages of the style branches becomes apparent at once. Cassini recognized the value of these hairs in sweeping out all the pollen from the dehiscent sacs, but a new emphasis is given to their efficiency, and an explanation is afforded of the development of rings of long hairs at or below the point of branching, as in types X and XI, Fig. 2, the spreading hairs at the apex of the style branches in types IV and V, and at the base of the appendages in types VII and VIII. The functions of the staminal appendages, hitherto obscure, become more obvious, for with the corolla-tube of a given length and the stamens in proportion, the amount of pollen produced can be reduced and the staminal tube remain the same length by the production of a membranous prolongation at the apex of each anther. This is a very simple method of reducing the polliniferous tissue while preserving the efficiency of the staminal tube in the pollen-presentation mechanism. The function of the basal appendages is also made clear, since a tube terminating in ten more or less hemispherical lobes, as in type 3, Fig. 1, could not be closed entirely by the style unless that organ actually entered the tube for some distance, in which case the pollen in that part of the sac past which the style had grown would be more or less lost for pollination purposes, unless it were swept up the tube by hairs situated lower down on the style. If the apex of the style merely reached to the lobes when the anthers dehiscent, some of the pollen would fall through the interstices to the bottom of the corolla-tube and thus be lost. If, however, the hemispherical lobes were prolonged into flattened auricles, or ciliate tails, or more elaborate appendages, the style with or without appendages could close the lower end of the staminal tube completely without encroaching on the polliniferous region, and thus no pollen would be wasted so far as the pollen-presentation mechanism was concerned.

The question immediately arises whether these conditions actually occur at the time of the dehiscence of the anthers. A considerable number of species has been examined, and nothing has been found which is incompatible with the above explanation of the functions of the appendages. Each case, however, must be considered separately because a conical style appendage of type VIII may be quite efficient in the absence of basal appendages to the stamens, and the figures in the tables bear this out in a very striking way for the Astereae, Heliantheae, and Helenieae. Much exact work remains to be done on this point, and it is purposed to make this and other problems subjects for further instalments of these studies.

The hypothesis that the appendages of the style branches and the

apical and basal appendages of the stamens are the expression of a tendency to economy of pollen, which is limited only by the biological necessity of providing sufficient pollen to ensure fertilization, implies that these appendages will show correlative development, and Tables I and II are an attempt to analyse the development of these structures throughout the order.

In such tables it is very difficult to assign the correct value to each genus, since a small genus may represent a large, almost extinct group, while a large genus may be composed of numerous small variations about a single type. It is obvious that a higher value should be given to one species of the former genus than to one of the latter. Likewise, if the genus is large and variable, it is proper that it should receive a proportionate value. Since the task of tabulating the characters of 13,000 species is out of the question, the genera have been analysed in the following way: If a genus shows one type of style or stamen it has been counted 1; if it shows two types, then each type has been counted  $\frac{1}{2}$ ; and if, as in some large genera, it shows three types, then each type has been counted  $\frac{1}{3}$ , so that a large and varied genus has the value  $1\frac{1}{3}$  in the tables. It was thought inadvisable to give a genus showing two types a value greater than unity in order to keep the numbers approximately those of the genera in each tribe.

#### DESCRIPTION OF STAMENS AND STYLES.

The types given here are in most cases of a synthetic character, as many of the intermediate stages between one definite type and the others occur. In all the types of stamens except types 1 and 4<sup>a</sup> the apical appendage is present. It is a more or less flattened outgrowth and nearly always simple in outline, very rarely bifid. In certain types this appendage becomes more elongated (types 6<sup>a</sup>, 11, 15, and 16), or, as in *Sclerolepis* (Fig. 5), it may become truncate, or it may have the apex inflexed, as in the Ambrosinae. Type 1, in which the apical appendage is absent, occurs only in the Piquerinae, a sub-tribe of the Eupatorieae; 4<sup>a</sup> is a rare type which occurs only in *Eleutheranthera*: it is type 4 without the apical appendage. Type 6<sup>a</sup> is interesting as occurring in a discoid genus of Senecioneae, *Culcitium*. This type and type 11, which occurs in the Mutiseae (*Leuceria*), are to be compared with the elongated apical appendages of types 15 and 16, which are the typical forms for the Cynareae.

The basal appendages show more variation. They are absent entirely from types 1, 2, and 3. In the other types the polliniferous region is shaded so that the appendages may be clearly distinguished. Type 4 shows a sagittate stamen with very small auricles; types 5 and 6 show these auricles enlarged: in the former they are obtuse, in the latter acute. Type 6<sup>b</sup> occurs very seldom: the auricles here are enlarged. Type 7 is a sagittate stamen with the auricles connate; type 8 shows the beginning of the



so-called tails, the auricles being mucronate; type 9 is similar, but the auricles are connate and mucronate; types 10, 11, and 12 show stages in

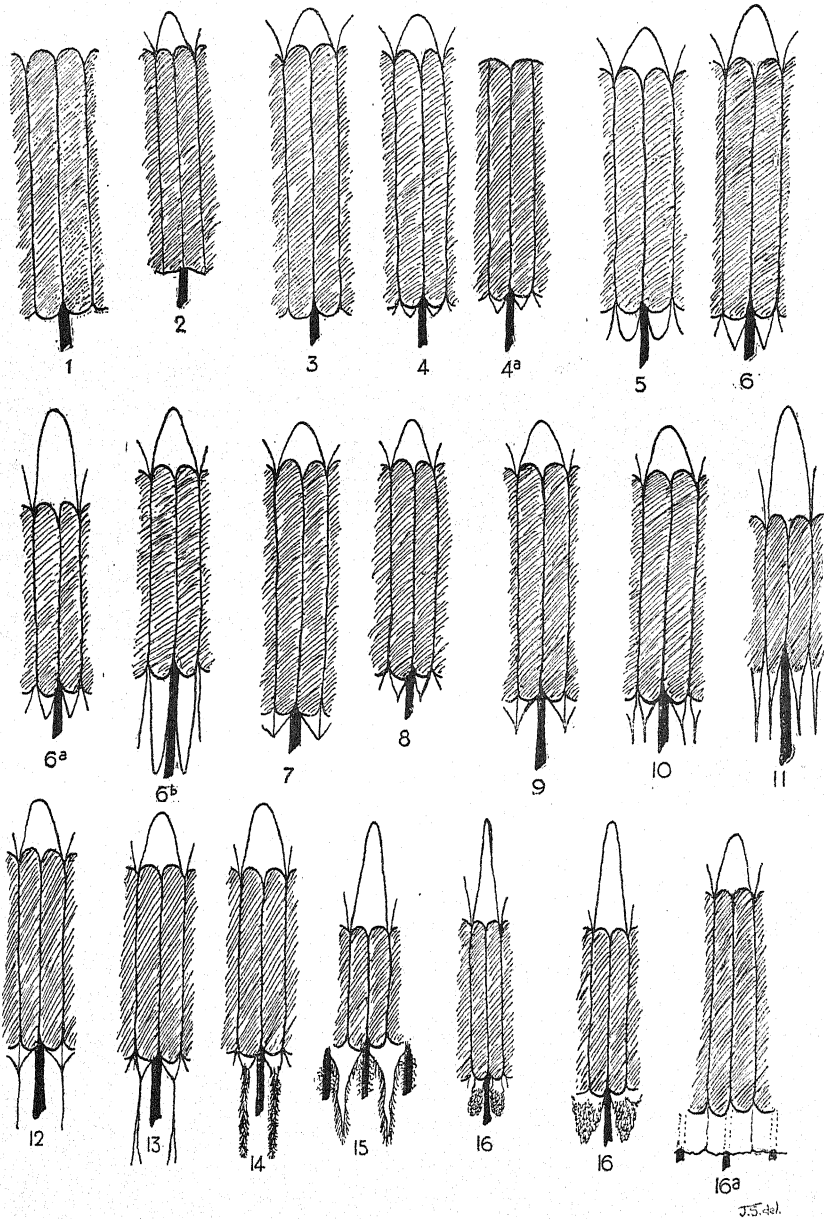


FIG. 1. Types of Stamens in the Compositae (for explanation see text, pp. 461-4).

the elongation of these tails, which remain simple and undivided; in type 12 the tails of the contiguous auricles are united; types 13 and 14 show further

elaboration of these appendages, the former being branched and the latter more or less lacerate or fringed with hairs; type 15 is similar to 14, but the tails of contiguous anthers are united. Type 16 includes two forms of

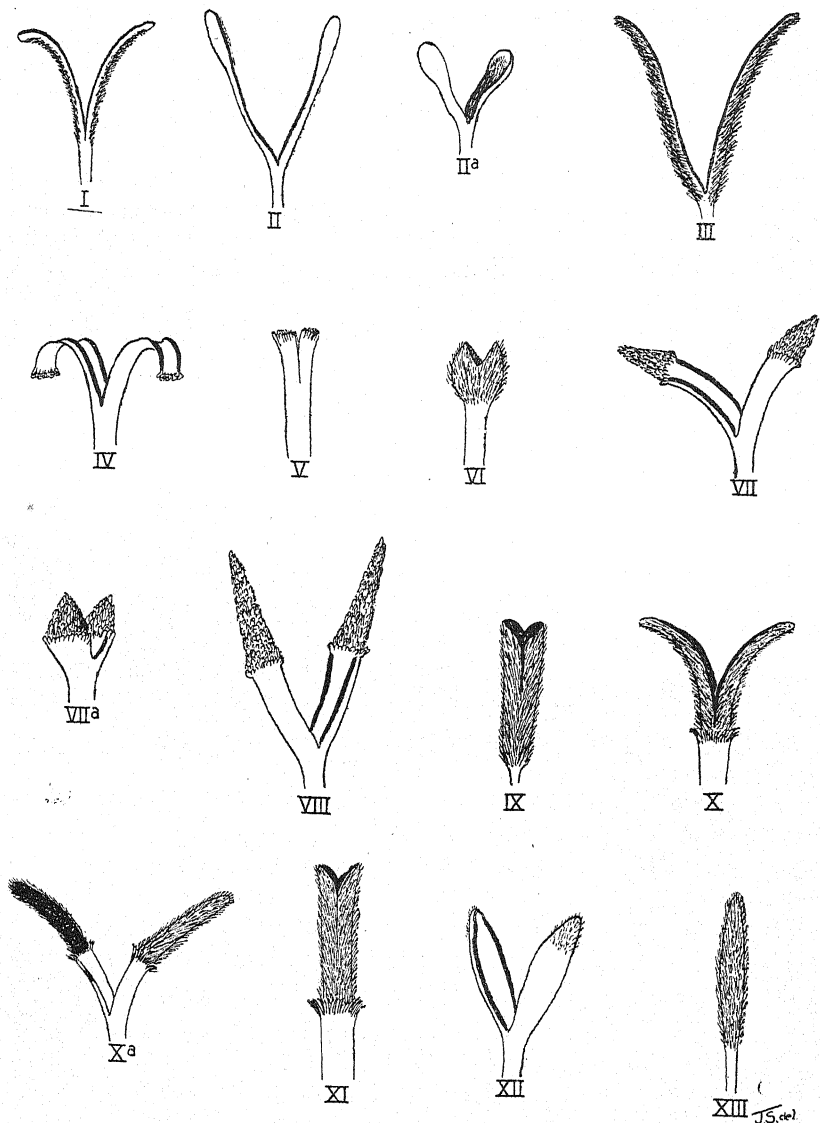
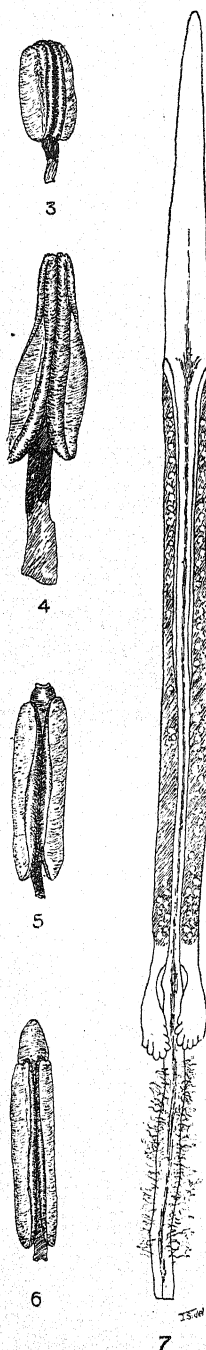


FIG. 2. Types of Styles in the Compositae (for explanation see text, pp. 464-5).

lacerate or more or less elaborate tails; the first form has the adjoining tails free and the other has them united; both these forms occur in many genera of the Cynareae. Fig. 7 represents an example of this type which should be compared with the forms shown in Figs. 3-6. As a final stage in



the fusion of the basal appendages, there is the form which occurs in *Tricholepis*, type 16<sup>a</sup>, where the appendages of each stamen are fused and those of neighbouring stamens connate, so that there is a continuous sheath of non-polliniferous tissue at the base of the staminal tube.

The very various style forms can be reduced to thirteen mean types, around which the numerous variations can be grouped. Type I is characteristic of the Cichorieae, and the female florets of most of the other tribes. The region of the stigmatic papillae is indicated by the dark line and extends from the apex almost to the point of branching. The external surface of the style branches is more or less hairy. Type II is characteristic of the Eupatorieae; the apex of the style branches is more or less clavate, the branches vary considerably in length, as they do in most of the types, and may be reduced to such a form as is shown by *Ophyrosporus*, type II<sup>a</sup>. Type III is characteristic of the Vernonieae; the style branches are more or less rounded and hirsute, with an obtuse apex. Type IV, as will be seen from Table II, is the most common form of style in the hermaphrodite florets. The style branches are flattened or more or less rounded, and may be longer or shorter than in the mean type; the stigmatic papillae are marginal and reach to the apex, which is truncate or rounded and more or less hairy. This type, when it occurs in the male flowers of the disc, usually remains closed and unbranched; this condition is represented in type V. Type VI is characteristic of the male disc florets in Calenduleae; it has the style branches very short and no stigmatic papillae.

Type VII is a form similar to type IV, but with the apex prolonged into a hairy, more or less conical or rounded appendage; the stigmatic papillae cease at the base of the appendage. This type may have the branches so short that a form arises like that of *Bellis*, type VII<sup>a</sup>. Type VIII is similar, with the appendage more elongated.

FIGS. 3-7. 3. *Piqueria serrata*.  $\times 18$ . 4. *Adenostemma viscosum*.  $\times 42$ . 5. *Sclerolepis verticillata*.  $\times 10$ . 6. *Eupatorium cannabinum*.  $\times 18$ . 7. *Centaurea scabiosa*.  $\times 18$ .

Type IX is characteristic of the Mutiseae and resembles type XI, without the ring of long hairs; here, as in the former type, fusion of the branches may be more or less complete, so that the stigmatic region is limited to a hollow, rounded region at the apex. Types X and XI are characteristic of the Cynareae, and are distinguished by the ring of long hairs situated at (type X) or below (type XI) the point of branching: in the former the stigmatic papillae extend from the point of branching to the apex; in the latter there is a very short zone of stigmatic papillae, but numerous intermediate stages are found in the fusion of the two branches above the ring of hairs. Type X<sup>a</sup> is the form of style which occurs in the female florets of many Cynareae; here the point of branching is below the zone of hairs and the stigmatic papillae extend from this ring to the apex. Type XII is characteristic of the Inuleae and has no appendages, the style branches being rounded and glabrous or slightly hairy externally, with the stigmatic papillae marginal and extending to the apex. Type XIII is an unbranched, club-shaped, hirsute form, common in the male disc florets of several tribes; it has no stigmatic papillae, and acts solely as a pollen-presenter.

#### DISCUSSION OF TABLES.

The types of stamens have been arranged so that the complexity increases from 1 to 16, and the order in which the tribes are given by Hoffmann has been altered so that the affinities may be rendered more diagrammatically clear. The chief alterations are the interchanging of the Vernoniaceae and Eupatoriaceae, the Mutiseae and Cynareae, and the placing of the Inuleae and Cichoriaceae nearer the Senecioneae.

The Eupatoriaceae are placed first because it is in the Piquerinae, a sub-tribe of this group, that the simplest type of anther occurs. The Piquerinae, which has the typical style of the Eupatoriaceae, is a group of eight essentially American genera. *Adenostemma*, however, has several Old World species, *A. viscosum* being very cosmopolitan. An interesting modification of the stamen of this sub-tribe occurs sometimes in *Adenostemma*. At a period when all the pollen grains have disappeared from the dehiscent anthers, the line of dehiscence is found to extend to a point a little below the apex (Fig. 4). This is a variation which, by preventing the untimely overflow of pollen from the staminal tube, would carry out the same function as a terminal, non-polleniferous appendage. In this connexion it should be noted that Bentham places this genus next to *Sclerolepis*, which he makes the first genus in the Ageratae, and *Sclerolepis* shows an abnormal truncate form of apical appendage (Fig. 5) which has all the appearance of a reduced type. The other genera of the Eupatoriaceae have the type of appendage shown in Fig. 6. The Vernoniaceae show a greater variety and have higher types of stamens, but this can be correlated with

| TRIBES     | TABLE I --- TYPES OF STAMENS |    |     |     |    |     |   |     |    |     |     |     |     |     |    |     |
|------------|------------------------------|----|-----|-----|----|-----|---|-----|----|-----|-----|-----|-----|-----|----|-----|
|            | 1                            | 2  | 3   | 4   | 5  | 6   | 7 | 8   | 9  | 10  | 11  | 12  | 13  | 14  | 15 | 16  |
| EUPATORIÆ  | 7                            | 1  | 30  |     |    |     |   |     |    |     |     |     |     |     |    |     |
| VERNONIÆ   |                              |    |     |     | 26 | 3   | 4 |     |    | 14½ |     |     |     |     | 1  |     |
| ASTEREÆ    |                              | 6½ | 85½ | 2½  | ½  |     |   |     |    | 1   |     |     |     |     |    |     |
| HELIANTHÆ  |                              | 10 | 72½ | 40  | 6½ | 1   |   |     |    |     |     |     |     |     |    |     |
| HELENIÆ    |                              | 2½ | 35  | 14½ | 2  | 2   |   |     |    |     |     |     |     |     |    |     |
| INULEÆ     |                              |    | 3½  | 1½  |    |     |   |     | 1  | 74  |     | 29½ | 22½ | 12  |    |     |
| ANTHEMIDEÆ | 2                            | 40 | 2½  |     |    |     | 1 |     |    |     |     |     |     |     |    |     |
| SENECIONEÆ | 2                            | 27 | 10½ |     |    |     |   |     |    |     |     |     |     |     |    |     |
| CALENDULEÆ |                              |    | 2   | ½   |    |     |   | ½   | 1  | 13  |     | 1   |     |     |    |     |
| CICHORIEÆ  |                              |    | ½   |     | ½  | 25½ |   | 33½ |    |     |     |     |     |     |    |     |
| ARCTOTIDEÆ |                              |    | 1½  | 2   | 3  | 2   | ½ | ½   |    | 2   |     |     |     |     |    |     |
| MUTISIEÆ   |                              |    | 1½  | ½   |    |     |   |     |    | 2   | 22½ | 2   | 2½  | 23½ | 1½ | 1   |
| CYNAREÆ    |                              |    |     |     |    |     |   |     | 5½ | ½   |     | 5   |     | 1   | 9½ | 12½ |

| TRIBES     | TABLE II --- TYPES OF STYLES |     |     |     |   |    |     |      |    |     |    |      |      |
|------------|------------------------------|-----|-----|-----|---|----|-----|------|----|-----|----|------|------|
|            | I                            | II  | III | IV  | V | VI | VII | VIII | IX | X   | XI | XII  | XIII |
| EUPATORIÆ  |                              | 36½ |     |     |   |    |     |      |    |     |    |      |      |
| VERNONIÆ   |                              |     | 40  |     |   |    |     |      |    |     |    |      |      |
| ASTEREÆ    |                              |     | 14  | 11  |   |    |     | 29½  | 45 |     |    | 1    | 5    |
| HELIANTHÆ  |                              |     | 7½  | 17  | 8 |    |     | 40½  | 26 |     |    | ½    | 31½  |
| HELENIÆ    |                              |     | 1   | 24½ | 1 |    |     | 19½  | 12 |     |    | 1½   |      |
| INULEÆ     |                              |     | ½   | 73  | 2 |    |     |      |    |     |    | 152½ | 18   |
| ANTHEMIDEÆ |                              |     |     | 42  | 4 |    |     |      |    |     |    |      |      |
| SENECIONEÆ |                              |     | 8   | 29  | 2 |    | 7   | 2    |    |     |    |      | 2    |
| CALENDULEÆ |                              |     |     | 2   |   | 15 |     | ½    |    |     |    |      | ½    |
| CICHORIEÆ  | 61                           |     |     |     |   |    |     |      |    |     |    |      |      |
| ARCTOTIDEÆ |                              |     |     | 8   |   |    |     |      |    | 2   |    |      |      |
| MUTISIEÆ   |                              |     |     | 32  |   |    |     | 1½   |    | 24½ |    | 1    |      |
| CYNAREÆ    |                              |     |     |     |   |    |     |      |    |     | 12 | 20   | 75   |

TABLES I and II.

the simple form of the style, whereas the Eupatorieae have both stamens and style simple.

In the remaining groups it will be seen that the stamen with an apical appendage but no basal appendage, type 3, is the most common form. This form is undoubtedly the fundamental type of stamen for the Compositae, types 1 and 2 being reductions and the others amplifications of it. The Astereae show this simple stamen in the large majority of the genera, but this tribe, while showing the simplest type of auricle in three<sup>1</sup> genera, also shows the truncate type, which is taken as a reduction form in twelve genera. The Astereae, therefore, are the antepenultimate step in the reduction series which ends in the Piquerinae. This type of anther occurs in a considerable proportion of the Heliantheae, but this tribe also shows a large percentage of higher forms. The Helenieae are very similar to the Heliantheae, while the majority of the Anthemideae have anthers of type 3. The Inuleae are notable on account of the majority of the genera showing anthers with relatively complex basal appendages, but this is to be correlated, as in the Vernonieae, with the simple style. The majority of the genera in Senecioneae remain relatively simple in their stamens. The Calenduleae, a small and specialized group in many ways, show a considerable proportion of genera with stamens of the higher types. With few exceptions the Cichorieae have stamens of type 6 or 8, both types occurring in most of the genera. The Arctotideae are very similar to the Senecioneae, but show type 10 in three genera, and thus form an intermediate stage between the Senecioneae and the Mutiseae, which latter tribe is very similar to the Inuleae in the degree of complexity reached by the anther appendages. The Mutiseae show types 15 and 16 in three genera, thus forming a transition to the Cynareae, the majority of which show one or other of these two complex types.

Considering the probable lines of development and omitting as negligible those cases where the number in the tables is less than two,<sup>2</sup> we find that the Senecioneae form a group from which radiate five lines thus: two short lines, one to the Anthemideae and the other to the Cichorieae; two main lines, the first leading to the Eupatorieae and giving off branches to the Helenieae, Heliantheae, Astereae, and Vernonieae, the second leading to the Cynareae and giving off branches to the Calenduleae, Arctotideae, and Mutiseae; the fifth leading to the Inuleae through the higher types in the Senecioneae.

Keeping these lines of probable development in mind, consider the figures of Table II, neglecting as before figures below two. The columns

<sup>1</sup> See explanation of tables, p. 461.

<sup>2</sup> Except in the Astereae, where parts of two large genera, *Olearia* and *Celmisia*, are included in the 1, and in Calenduleae, where parts of the largest two genera, *Osteospermum* and *Tripteris*, are included in the 1 of the table.

under types V and XIII may be omitted, as these types of styles occur wherever there is the necessary form of floret, as does also type I. The Eupatorieae and Vernonieae have simple styles. It will be seen at a glance that type IV is the form of most common occurrence, and this type is undoubtedly the fundamental type of style for the Compositae. The majority of the Astereae show styles with appendages more or less complex, and with this is to be correlated the great preponderance of simple stamens in this tribe. The Heliantheae show a very similar stage of development, but the Helenieae have a distinctly larger proportion of genera of the simple type IV.

In the Anthemideae the great majority of genera have this type of style. The Inuleae are again anomalous in having a peculiar style, type XII, which is almost, if not quite, as simple as type IV, and is to be correlated with the preponderance of the higher types of stamens in this tribe. The Senecioneae, while showing a considerable range of structure in the style, have a large proportion of type IV. Indeed, Bentham describes type IV as the characteristic style of the Senecioneae. The Calenduleae have quite a special type of style, type VI. The Cichorieae, without exception, have the style of type I, which is the type for the ray florets in many other tribes. As with the stamens, the Arctotideae show a considerable percentage of simple styles with some of the higher types, forming a transition to the Mutiseae, and the latter tribe forms an ideal intermediate stage between the Arctotideae and the Cynareae, which have the highest form of style, types X and XI.

Neglecting columns V and XIII for the above-mentioned reasons, and taking type IV as a base, we find that lines of development are to be found almost diagrammatically similar to those in Table I. As before, there are two short lines, one to the Anthemideae and the other to the Cichorieae; two main lines, the first leading to the Eupatorieae and giving off branches to the Helenieae, Heliantheae, Astereae, and Vernonieae, the other leading to the Cynareae and giving off branches to the Arctotideae and Mutiseae; and a fifth leading to the Inuleae. The only difference is that a sixth line leads to the Calenduleae, the style of which cannot be considered a transition stage to that of type IX.

Thus from these tables alone we get two phylogenetic diagrams almost identical. This is only to be expected if the pollen-presentation mechanism has undergone progressive or retrogressive development, and economy of pollen seems to be the simplest explanation of this progressive elaboration of those parts which deal with the problem of delivering to each insect visitor a sufficient, but also a minimum, amount of pollen.<sup>1</sup>

<sup>1</sup> To those who have watched the miserly way in which the pollen-presentation mechanism of *Centaurea* supplies pollen to the bees the idea of economy is sure to have suggested itself, but, of course, there is in this case the additional mechanism of sensitive stamens.

The pollen-presentation mechanism is not the only character which shows progressive variation in the Compositae, and any phylogenetic scheme must take into account the form, development, and colour of the corolla, the form of the pappus, the composition of the capitulum, and the geographical and geological distribution of the main divisions of the order; the available data concerning these characteristics must be increased before a satisfactory discussion of the inter-relationships of the tribes can be attained.

#### CONCLUSION.

In dealing with so recent an order, and one which, as a whole, is herbaceous, the fossil evidence is scanty and conflicting. Such being the case, the somewhat scattered fossil literature of the order awaits critical study.

That the cytological phenomena might be of value occurred to the writer early in 1913, and material was afterwards prepared for cytological investigation. Since that time the question of correlation between chromosome dimensions and phylogeny has been the subject of several papers (14 and 7) which have dealt with the problem in relation to the larger groups of the animal and plant kingdoms, and the results already obtained are being held over, pending a critical study of a considerable number of closely related species. The characteristic more or less spherical forms of the chromosomes in most Compositae may be mentioned as one interesting fact, in view of the controversial condition of the subject at present.

In addition to the data which have already been collected concerning the floral organs, a number of isolated but relevant observations have come under my notice during the four years which I have been working at this problem, and although these cannot be introduced readily into such a brief and tentative account as the foregoing, all the known facts are quite compatible with the origin of the tribes which is suggested as a result of the analysis of the staminal and stylar forms.

Acknowledgements are due to the Regius Keeper of the Royal Botanic Garden of Edinburgh for permission to study in the herbarium and for fresh material of many species of *Senecio*; to Professor M. C. Potter, for access to books and periodicals; to Professor A. C. Seward, for help with the fossil literature; to Professor A. H. Trow, Mr. W. Hales, A.L.S., Chelsea Physic Garden, and Mr. R. Irwyn Lynch, M.A., University Botanic Garden, Cambridge, for material.

#### SUMMARY.

1. The hypothesis that the appendages of the style branches and the apical and basal appendages of the anthers are the expression of a tendency



to economy of pollen, which is limited only by the biological necessity of providing sufficient pollen to ensure fertilization, is supported by evidence of correlative development of these appendages.

2. Tables are given showing the relative frequency of occurrence of the different types of styles and stamens in the various tribes, and these tables are also used to show lines of development and specialization in the pollen-presentation mechanism.

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# Meiotic Divisions in the Microspore Mother-cells of *Smilacina racemosa* (L.), Desf.

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With Plate XXII and one Figure in the Text.

BECAUSE of some remarkable statements made by Lawson ('11 A) concerning 'The Phase of the Nucleus known as Synapsis', material was collected and work on this paper was begun. Cytology has for many years had many questions of dispute among its investigators, but it is only through continued investigation that the whole truth can be known. As has been found, *Smilacina racemosa* offers a favourable plant for study, as the flowers are borne in rather small, compact racemes, and several stages in the division of the microspore mother-cells may be found in one flower cluster, the older ones being at the base and the younger at the apex. McAllister ('13) used this plant for his study, but the species of *Smilacina* used by Lawson ('11 A and '12) was not indicated.

## MATERIAL AND METHOD.

Materials for this investigation were collected from the west side of a deep ravine north-east of Bloomington, Indiana, in the latter part of April and first of May in the years 1912 and 1913. The strong chrom-acetic solution and the stronger Flemming's chrom-osmic-acetic solutions were used in fixing the material. Whole racemes, or portions of racemes, were embedded in paraffin, and sections were prepared varying in thickness from 5 to 15 microns. Preparations were stained in Haidenhain's iron-alum-haematoxylin and in the regular triple stain, using orange G in aqueous solution or as a saturated solution in clove oil. Especially good preparations, showing metaphase and closely-related phases, were secured by using on material fixed in chrom-acetic a solution of gentian violet and clove oil, as suggested in the laboratory by F. L. Pickett.<sup>1</sup>

<sup>1</sup> A supersaturated solution of gentian violet in clove oil is prepared by adding to a saturated solution of the stain in absolute alcohol an equal volume of clove oil and allowing the mixture to stand in an open dish at room temperature until all the alcohol has evaporated. The resulting solution is then filtered through paper. After the safranin has been washed from the sections

As an aid in determining the true form and arrangement of chromatin in the second contraction, and segmented stages, nuclei were reconstructed in plastina, in order to facilitate the interpretation of the individual chromosomes.

The *Smilacina* which Lawson ('11 A and '12) used was probably *Maianthemum Convallaria*, Roth, as described in 'British Flora', fifth edition (1887), Bentham and Hooker, or the same plant as described under *Unifolium canadense* (Desf.), Green, or *Smilacina bifolia*, Desf., in Britton and Brown, second edition (1913). The plant used for this study is *Smilacina racemosa* (L.), Desf., as described in Gray's Manual, seventh edition (1908), or the same plant as is described as *Vagnera racemosa* (L.), Morung, in Britton and Brown, second edition (1913).

#### STATEMENT OF THE PROBLEM.

An attempt has been made in this investigation to make a very careful study of some of the stages in the meiotic divisions of the pollen mother-cells as found in *Smilacina racemosa* (L.), Desf. There has been much controversy for several years about some phases of cell activity, and, as Farmer ('12) says, 'there is still room for more light'.

The questions of the identity of the chromosomes throughout meiosis and of the behaviour of chromatin threads previous to and during synapsis have been much discussed in cytological literature, and there is still much diversity of opinion in regard to these questions. Among the other questions with which this paper deals are: the character of the synaptic ball, its position and relation to the size of the nuclear cavity, the nature of the chromatin thread after synapsis, during the spireme stage, and in the second contraction, the manner in which the thread segments, and the formation of the bivalent chromosomes.

*Resting Stage.* During the so-called resting stage the chromatin granules are arranged irregularly on fine linin threads throughout the nuclear cavity, giving the appearance of a network. The granules are more or less irregular in size, shape, and form. The appearance of a network is, no doubt, partly due to the overlacing of threads, as suggested by Lawson ('11 A), but there seem to be more crossed threads than there would be were this entirely the case. The fact that some portions of the fine linin thread

to just the intensity wanted in the finished preparation, the slide should be rinsed hurriedly with absolute alcohol and then covered with the clove oil violet. The violet should be allowed to act 20 minutes to 3 hours, although in some cases staining for 6 hours has given good results. It is then washed off with benzole or xylol and replaced with clove oil orange G. If staining of walls is not wanted, final differentiation may be secured by using pure clove oil after 10 to 15 minutes' use of the orange G solution. It has been found well to remove the clove oil orange G completely with flowing benzole or xylol and to mount the specimen from that medium. It is essential that no alcohol be allowed to come in contact with the sections after the use of the clove oil violet.

seem to be drawn in towards the chromatin granules as nuclear activity proceeds would also indicate that the network arrangement is not merely a fanciful one. There is nothing to indicate that there is a certain fixed number of threads in the resting stage, corresponding to the diploid number of chromosomes as stated by Lawson ('11 A and '12). Very few free ends were seen in nuclei showing this stage, and it would be impossible to count such individual threads if they were present as such in this stage (Figs. 1 and 2). The number of chromatin granules greatly exceeds the number of chromosomes in any stage of the division; so there is nothing in *Smilacina racemosa* to indicate that each chromatin mass is a prochromosome, as is claimed by several authors and as emphasized by Stout ('12) for *Carex aquatilis*. If the chromosomes do retain their identity throughout the resting stage, this identity is, at least, not recognizable at this stage.

Lawson ('11 A and '12) and McAllister ('13) show portions of the chromatin thread pairing during these early stages. Figs. 1, 2, 3, 4, and 5, Pl. XXII, of my preparations show similar stages to those figured by Lawson and McAllister, but in such nuclei, where there are so many crossings of the threads, or where the nuclear contents are in such a finely divided condition, it does not seem to be of any special significance that one portion of the thread should run along parallel to another portion for a short distance. It does not necessarily follow that there is any special relation existing between them. The irregularly shaped chromatin granules fuse later and finally form a smooth-edged thread. The fact that a few of the granules may fuse side by side does not necessarily establish the conclusion that there is a general side-by-side pairing throughout the whole nucleus, and, if such a phenomenon were characteristic of this stage, it would appear more often and in greater regularity. From the conditions as seen during this investigation, it is not possible to agree with Lawson ('11 A) that 'the chromatin threads are undoubtedly double from the beginning', nor that a definite pairing of threads takes place at this stage, nor that 'the developing spireme was not composed of a single continuous thread, but of a number of double threads, and the number corresponds with the diploid number of chromosomes . . . which become differentiated later'. McAllister maintains that there is no chromatin aggregation into prochromosomes.

As to the units which go together to make up the chromatin thread, it is hard to identify them. We cannot recognize the different hereditary characters which develop in the mature individual, in the nuclei of the spore mother-cells, and we cannot say with much degree of certainty that it is thus or so. A mature individual has too many characteristics for each of them to be bound up in a separate chromosome, and this is one argument (Farmer '07) given for the chromomere as the unit. The colloidal nature of the chromatin thread makes it hard to differentiate portions of the thread into units, when they are shifting or changing appearance as much as the

granules change in the chromatin thread. Lawson ('12) says that 'although threads may appear vacuolated, granular, or even beaded, they are composed of uniform material'. As the nucleus prepares for the activity of the division to follow, the lumps in the thread appear to become somewhat larger and to elongate so that each joins with its neighbours (Fig. 3).

Some of the thin linin threads which appeared to stretch across to other portions of the chromatin now appear to be drawn in and to help make the thread appear somewhat wider. Fig. 4 is taken from a section cut 5 microns in thickness and portions of the chromatin network are shown. Fig. 5 is a portion of another nucleus in which the chromatin granules are more evenly distributed along the thread, and it has more the appearance of a continuous thread than of a network. In some places portions of the thread lie almost parallel with each other. In one place two portions seem to be joined together by a slight attenuation of one thread, but it cannot be said with certainty that this indicates any pairing which will persist. The chromatin thread here is becoming slightly contracted.

The mother-cells, during the resting stage, are closely packed together and have thin walls and uniformly dense cytoplasm. From one to three or four nucleoli are seen irregularly placed in the nucleus, in among the irregular chromatin thread. The nuclear membrane appears as a sharp line separating cytoplasm from nuclear contents. The enlargement of the nuclear cavity, which takes place about this time, is doubtless coincident with an increase in the amount of containing fluid as Lawson ('11 A and '12) shows, but there is also, at this time and following it, a shortening and thickening of threads and a contraction which results in the diminution of the chromatin mass.

*Synapsis.* Measurements were taken of nuclei in the resting, synaptic, spireme, and segmented stages, and the results that were found do not substantiate Lawson's theory ('11 A and '12) that the nuclear cavity enlarges but the chromatin mass remains stationary in volume. The measurements show an increase in the size of the nuclear cavity just preceding and during synapsis, which size remains relatively stationary during the spireme and segmented stages; but unquestionably a decrease in the size of the chromatin mass is found in synapsis. In taking measurements nuclei were chosen at random, care only being taken that the section should be cut as nearly through the centre of the nucleus as possible. Of the nuclei measured, the average for the resting stage was 16 microns by 14 microns. Nuclei in the synaptic state were measured, with an average size of 23 microns by 18 microns, while the chromatin mass from these same nuclei gave an average measurement of 13 microns by 10 microns. The two dimensions denote the greatest and shortest diameters of each nucleus or mass. The measurements of the nuclei in spireme and segmented stages show sizes tallying with the measurements of the nuclear cavities of nuclei with chromatin in

synaptic state. These results show that there is an increase in the volume of the nuclear cavity, and also a decrease in the space which the chromatin mass occupies. Figs. 5, 6, 7, and 8 show progressive steps in the arrangement of the chromatin to form the synaptic mass.

Many cases were found in which the mass of contracting or contracted chromatin threads was fastened or swung to the nuclear membrane by fine strands as shown by Mottier ('07) and others, and is here shown in Figs. 6, 7, and 8. The position of the synaptic mass in the nuclear cavity seems to have no special significance, as the ball is located differently in different cells of the same loculus. The most common position was found to be close to one side of the cavity (Fig. 8), but other angles of sectioning would of course show masses in the same relative positions differently. There is no special position relative to gravity.

Fig. 6 shows the chromatin mass drawn away from the nuclear membrane, and its position is probably due partly to the expansion of the membrane, and partly to the contraction of the chromatin contents. Here the chromatin appears as lumpy portions of threads, which, when viewed from the standpoint of the stages preceding it, are due to the running together of some of the granules and the contraction of the whole thread. Some portions of the thread seem thicker than others, as McAllister ('13) has found, but no real pairing is apparent. Fig. 7 shows a slightly later stage, in which the chromatin appears balled up around the large nucleolus, and the threads are becoming more uniform in thickness. Some portions of the thread are drawn out from the mass by the strands which connect them to the nuclear membrane. Cut ends are shown where the knife has sectioned what were probably loops extending from the mass. These loops are not made up of double threads. Figs. 11 and 12 show tangential sections of late synaptic stages, and show loops and places where portions of the thread run along parallel to each other or in close proximation. They appear, however, as separate portions of thread rather than as portions of a double or paired thread. Figs. 9 and 10 were drawn from cells in the same loculus and side by side, with cell-walls not yet separated. They are typical stages showing the chromatin coming out of synapsis, but show nothing that indicates any pairing or previous pairing of threads. Fig. 13 shows a slightly older stage, and was found in a loculus with other nuclei in which the spireme threads were evenly distributed.

It cannot be said with certainty that there is a fusion of maternal and paternal chromatin in the synaptic state, because there is no means of distinguishing between parts of the chromatin on any such basis. Because of the fact that we can see the partial contraction of the chromatin into thread-like portions before they enter the synaptic state, and as tangential views show this condition persisting, there seems to be no indication that synapsis is other than a contraction of the chromatin and a subsequent shortening

and thickening of the threads to make a spireme of uniform thickness. Lawson ('12) maintains that there is a shortening and thickening of the spireme threads, but he does not show the chromatin aggregated into a tight synaptic ball. It is difficult to say just what position the chromatin units take, if there are any which are truly such. The significance of any special arrangements which may occur is also difficult to determine. It is not until later stages that true splits are seen in the chromatin thread (Figs. 16 and 17).

Figs. 11 and 12 show portions of thread which are much narrower than in most nuclei at this stage. Anthers from different flowers, however, show this difference in width of threads, especially noticeable at this stage. (Compare Figs. 9 and 10 with Figs. 11 and 12.)

*Spireme.* As the chromatin thread comes out of the synaptic state it is seen to have a greater diameter and is more uniform in thickness than the thread as it began to contract. This shortening and thickening has resulted in one continuous spireme. Sometimes the thread appears lumpy, but when it is evenly distributed throughout the nucleus, it appears as a smooth thread of uniform thickness (Fig. 14). Fig. 13 shows a slightly younger stage from a nucleus which was cut tangentially and was found in a loculus in which the nuclei all showed threads not yet fully untangled from the synaptic condition. The chromatin thread winds in and out through the nuclear cavity in a tortuous manner.

There has been much discussion as to whether the nucleus at this stage contains one continuous chromatin thread or as many threads as the diploid number of chromosomes. Lawson ('11 A) identified individual chromosomes throughout all the prophases of the nuclei of *Smilacina*, while McAllister ('13) finds a continuous spireme in *Smilacina racemosa*. This study shows the thread to be continuous, as has been found by McAllister ('13), Mottier ('07, '09, '14), and others. Several hundred nuclei were examined and studied under the best conditions to determine this point, and a model was made and sectioned to aid in the determination. Ends can be seen in abundance in sections of the nucleus showing spireme threads, but when it is possible to examine a whole nucleus or most of a whole one in one section the number of ends diminishes, so that it cannot be that there are as many threads as there are chromosomes appearing later. Fig. 14 shows almost a whole nucleus, and all the ends seen were carefully focused upon and appeared in such a plane that they surely were, at least most of them, cut ends. In Fig. 14 ten ends show, but this number is much too few if twenty-four is the haploid number of chromosomes for the plant. Long portions of continuous thread may also be traced through the nucleus.

Loops appear to reach out to the nuclear membrane of some nuclei in the spireme stage, and some portions of thread run along close to the membrane for short distances, indicating that there are probably some connexions existing between the thread and the membrane. Such stages as

are shown in Figs. 6, 7, 8, 9, and 10, and those shown in later stages (Figs. 15 and 16), would indicate that portions of the threads are attached to the nuclear membrane throughout a greater part of the prophases of the first division.

Some nuclei at this stage show jagged spireme threads, but most of the evenly distributed spiremes seem to be of almost uniform thickness. No splits were observed in the spireme at this evenly distributed stage, and not until later was any longitudinal split observed (Figs. 16 and 17).

*Second Contraction.* From the spireme stage the chromatin thread undergoes a second contraction, as has been observed by many observers (Fig. 14). The thread, in its shortening process, is drawn up in a tangled mass near the centre of the nucleus (Fig. 16). Radiating loops may be seen extending from the central mass and fastened to the nuclear membrane. Mottier ('07) found such a condition especially common in *Lilium*. As Lewis ('08) found in *Pinus*, in *Smilacina racemosa* 'the spireme often presents an extremely jagged structure just before cross-segmentation'. In some nuclei attenuations from portions of the bivalent chromosomes may be seen after segmentation (Fig. 18). Many nuclei showed much more jagged threads than are figured in the drawings accompanying this paper. During this stage and later the chromatin thread appears split (Figs. 16 and 17), and this fission is undoubtedly in preparation for the splitting of the chromosomes, which brings about the division of chromatin in the second division or formation of the granddaughter cells.

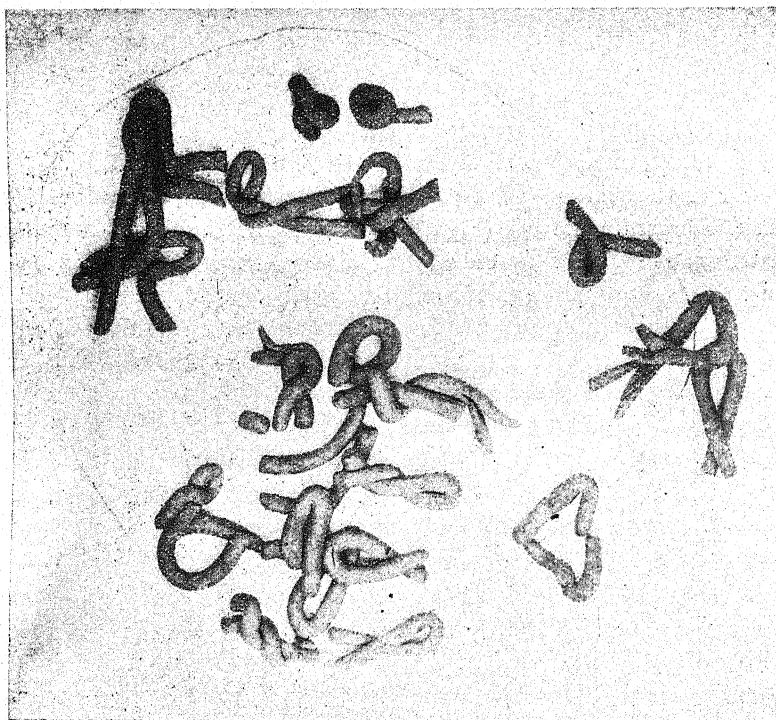
*Segmented.* Cross-segmentation of the spireme thread takes place while the spireme is in the state of second contraction. The thread may break near the periphery of the mass or nearer the centre, but it segments, and the bivalent chromosomes result from the approximation of two segmented portions of thread (Figs. 17, 18, 19, and 20). These findings agree with the descriptions of this stage as given by Farmer ('05), by Mottier ('07, '09, and '14), Lewis ('08), McAllister ('13), and several other cytologists, in contrast with those who maintain the view of parasynapsis taking place in the synaptic phase (Lawson '11 A and '12, Stout '12, and others). The spireme in *Smilacina racemosa*, however, is continuous or approximately so, and cross-segmentation takes place during the phase of the shortening and thickening of the thread just at the close of the second contraction stage. Lawson ('12) contends for the lateral pairing of the chromosomes which have retained their identity throughout the prophases, but states that the pairing is only temporary, so that it is not significant whether this pairing is lateral or end to end. We do not know just what is the true significance of this association, but it is in preparation for the reduction which takes place in the formation of the daughter nuclei. The point of real significance is whether or not there is a pairing of the somatic chromosomes in the earlier prophases which brings about a union of maternal and paternal chromatin.

A photograph was taken of one of the plastina models made to aid in



the determination of the true forms and shapes of chromosomes, and it was found that the photograph bears a striking resemblance to the nucleus as seen in the microscope and as figured with drawing accompanying this paper. (Compare Text-fig. with Fig. 17.)

The bivalent chromosomes which result from cross-segmentation continue to shorten and thicken until they take the form as shown on the spindle plate (Fig. 21). They can be found assuming all the different shapes which have been described for them and in many different arrange-



TEXT-FIG. Photograph of plastina model of the same nucleus from which Fig. 17 on Pl. XXII was drawn.

ments. Figs. 16 to 20 show different shapes in which the chromosomes appear during this segmented stage. Some nuclei show bivalents of different lengths and widths (Figs. 17, 18, 19, and 20). Fig. 18 shows one bivalent which is bent back upon itself. Fig. 19 shows one portion of the segmented thread which is curved twice, but the limbs are not yet tightly wound about each other (Figs. 17 and 18), while others remain in ring-shaped forms.

Farmer and Digby ('13) have discussed the possible significance of the different sizes of chromosomes and the constancy of this variance in any one

species, and attach no special significance to this difference in size, as some cytologists do. The sizes do not remain constant throughout any one species; so no hereditary significance can be attached to these differences. In *Smilacina racemosa* different sizes of chromosomes appear in the segmented stage and on the spindle plate, but the differences in size and shape are not constant: that is, a chromosome of certain size or shape cannot be found in all nuclei at this stage, and the chromosomes are constantly changing size as they contract.

Lawson ('11 A) gives 20 as the probable haploid number of chromosomes in the *Smilacina* with which he worked, but later (Lawson '12) changed the number to 14. McAllister ('13) gave 24 for the haploid number in *Smilacina racemosa*. The findings in this investigation agree more nearly with McAllister, the countings showing from 20 to 24 chromosomes.

The cell-walls are thin in the resting stage, but, as the cells round off during and immediately after the synaptic stage, they begin to thicken, and in the segmented and spindle stages a very thick special wall surrounds the pollen mother-cells. (Compare Figs. 1, 7, 14, 15, 20, and 21.)

*Spindle.* The chromatin thread undergoes a continuous shortening and thickening throughout the prophases, and, when the chromosomes appear on the spindle in the metaphase, they are very short and thick (Fig. 21). There is no indication, in the nuclei examined in this investigation, that the spindle fibres are formed by a gradual contraction of the nuclear membrane, a closing in around each chromosome and a subsequent tension resulting in the cytoplasm forming fibres, as advocated by Lawson ('11 B) for the microspore mother-cells of *Disporum gladiolus*, *Yucca*, *Hedera*, for the vegetative cells in the root-tip of *Allium*, and later for the microspore mother-cells of *Smilacina*. The difficulties involved in such a process have been discussed by Farmer ('12 and '13), and further discussion seems useless here. In no case was the nuclear membrane in *Smilacina racemosa* seen to contract around the chromosomes in such a manner as Lawson described. The spindle fibres, rather, appear to be formed in the manner described by Mottier ('97) and others. It was often found that in the same loculus were nuclei in the typical segmented stage and other nuclei with fully developed bipolar spindles. In some nuclei the nuclear membrane appeared to be partly broken down, in some fibres were appearing, in others portions of the membrane persisted as fibres appeared, while in still others the membrane was entirely broken down and the chromosomes were arranged on the spindle in the typical metaphase condition.

#### SUMMARY.

1. The microspore mother-cells of *Smilacina racemosa* have the nuclear contents in a finely divided state during the resting stage, with irregularly shaped granules held in the meshes of a fine linin network.

2. There is no lateral pairing of the chromatin threads during this or the synaptic stage. Neither does the chromatin content consist of a number of chromatin threads which equals the diploid number of chromosomes for the species.

3. Synapsis is a stage which is characterized by a very general contraction of the nuclear contents into a tight ball, usually lying at one side of the cavity, and an increase in the size of the nuclear cavity.

4. The contents of the nucleus are often connected with the nuclear membrane by fine strands.

5. The mass of chromatin threads untangles, and a continuous spireme of uniform diameter is evenly distributed throughout the nuclear cavity. Connexions between the chromatin thread and the nuclear membrane still persist.

6. Immediately after this state of the evenly distributed spireme follows a second contraction or central entangling of the spireme with loops radiating from the centre to the periphery. Longitudinal splits may be seen in the thread at this time.

7. Cross-segmentation of the chromatin thread takes place either at the centre or near the periphery of the tangle, and a lateral approximation of the limbs of loops or of separate portions of chromatin thread takes place to form the bivalent chromosomes.

8. Fibres appear around the nucleus, the membrane breaks down, the characteristic bipolar spindle is finally formed, and the bivalent chromosomes are arranged in a plate at the equator of the spindle.

I wish to express my gratitude to Prof. D. M. Mottier for his most helpful suggestions and criticisms during the preparation of this paper.

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## EXPLANATION OF PLATE XXII.

Illustrating Miss Woolery's paper on Meiotic Divisions in the Microspore Mother-cells of *Smilacina racemosa* (L.), Desf.

All figures were drawn from sections with the aid of the Abbe camera lucida with Zeiss apochromatic immersion 2 mm. apert. 1.40, and compensating ocular 12. Magnification about 1750 to 1800.

Fig. 1. Resting nucleus of the microspore mother-cell, showing typical structure of nucleus and cytoplasm.

Fig. 2. Resting nucleus with slightly larger chromatin granules. Some portions of threads lying parallel.

Fig. 3. Tangential view, showing larger lumps of chromatin. Some portions of the linin thread have been drawn in.

Fig. 4. Tangential view of stage similar to Figs. 1, 2, and 3.

Fig. 5. Chromatin thread beginning to contract and to become more uniform in diameter.

Fig. 6. Enlargement of nuclear cavity and contraction of nuclear contents. Strands are connecting chromatin with the nuclear membrane.

Fig. 7. Chromatin contents much contracted and nuclear cavity much enlarged. Strands extend from chromatin to nuclear membrane. The cell-wall is somewhat thickened.

Fig. 8. A typical tight synaptic ball.

Fig. 9. Nucleus, showing untangling of synaptic ball, with thread of almost uniform thickness.

Fig. 10. Slightly older stage. Several cut ends are visible. Figs. 9 and 10 are drawn from adjoining nuclei in the same localus.

Fig. 11. Tangential view of nucleus just after synapsis, showing looping and twisting of thread.

Fig. 12. Tangential view similar to Fig. 11. The thread is much narrower than in most nuclei at this stage.

Fig. 13. Untangling not yet complete. Many cut ends are shown. Thread of uniform diameter.

Fig. 14. Typical spireme stage. Relatively few cut ends to be found.

Fig. 15. Segmentation into bivalent chromosomes has just taken place. Some bivalents are formed by approximation of limbs of loops and some by the approximation of the separate chromosomes.

Fig. 16. Second contraction stage. Longitudinal splits of the thread are appearing. Looping and twisting of threads is taking place.

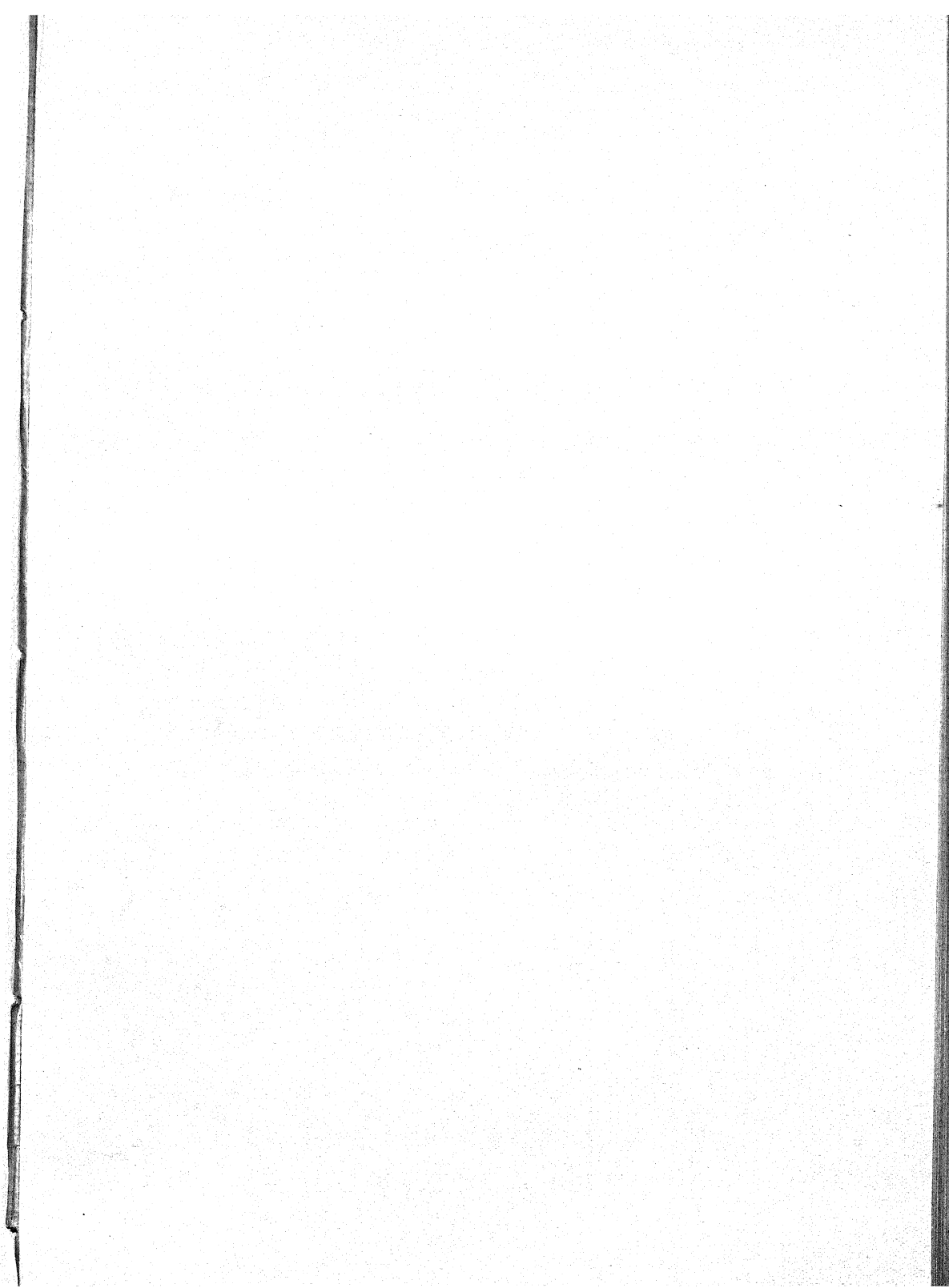
Fig. 17. Bivalent chromosomes in various shapes and forms. (Cf. photograph of model on p. 8.)

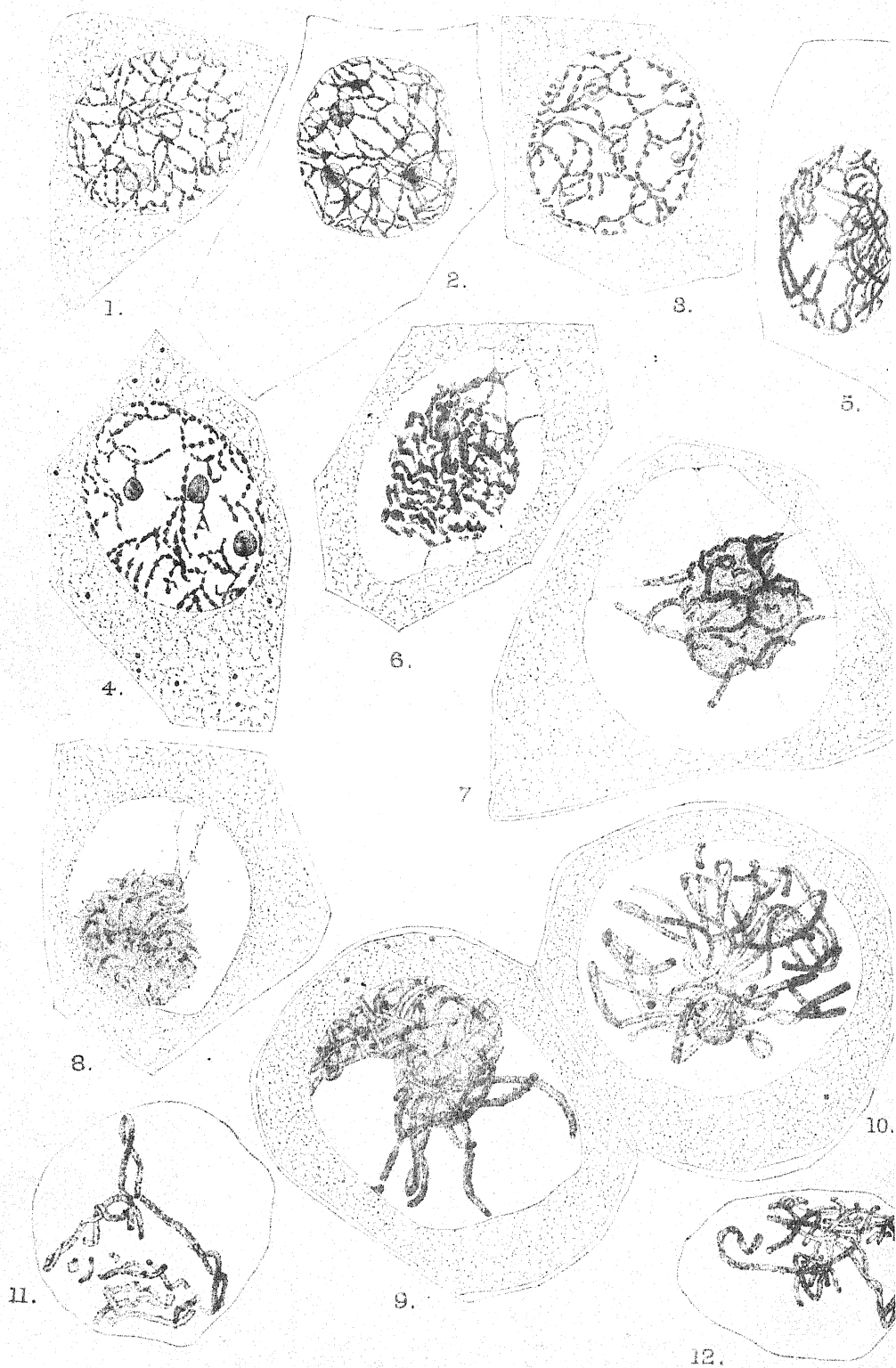
Fig. 18. Segmented stage. The bivalent chromosomes are of different sizes. One chromosome of each of three bivalents is drawn out as if by some tension.

Fig. 19. Bivalent chromosomes, one with two loops but with halves not yet twisted around each other.

Fig. 20. Various shapes of bivalent chromosomes.

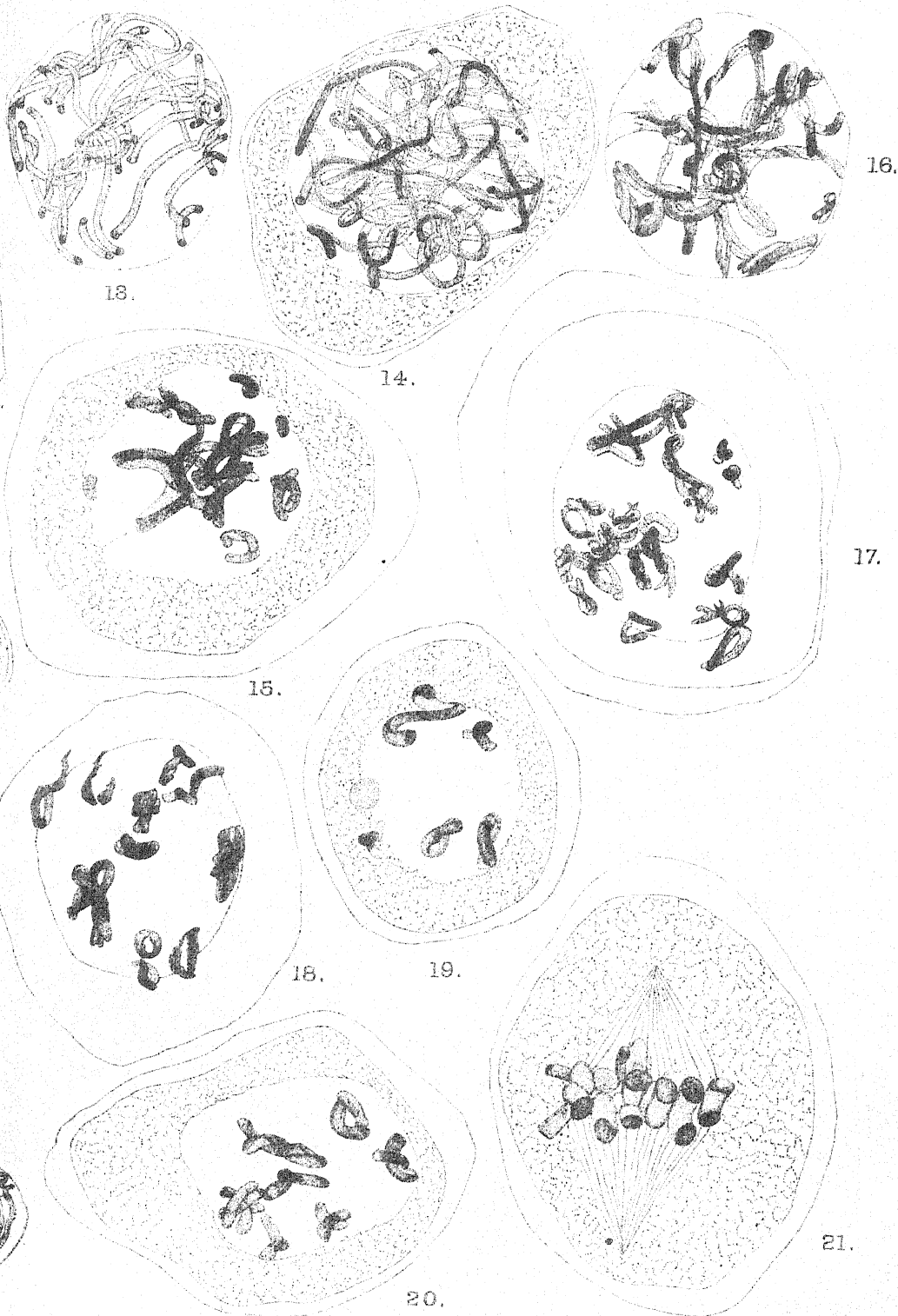
Fig. 21. A typical bipolar spindle showing metaphase. The cell-wall is very thick.





R.W. del.

WOOLERY — *SMILACINA RACEMOSA*.



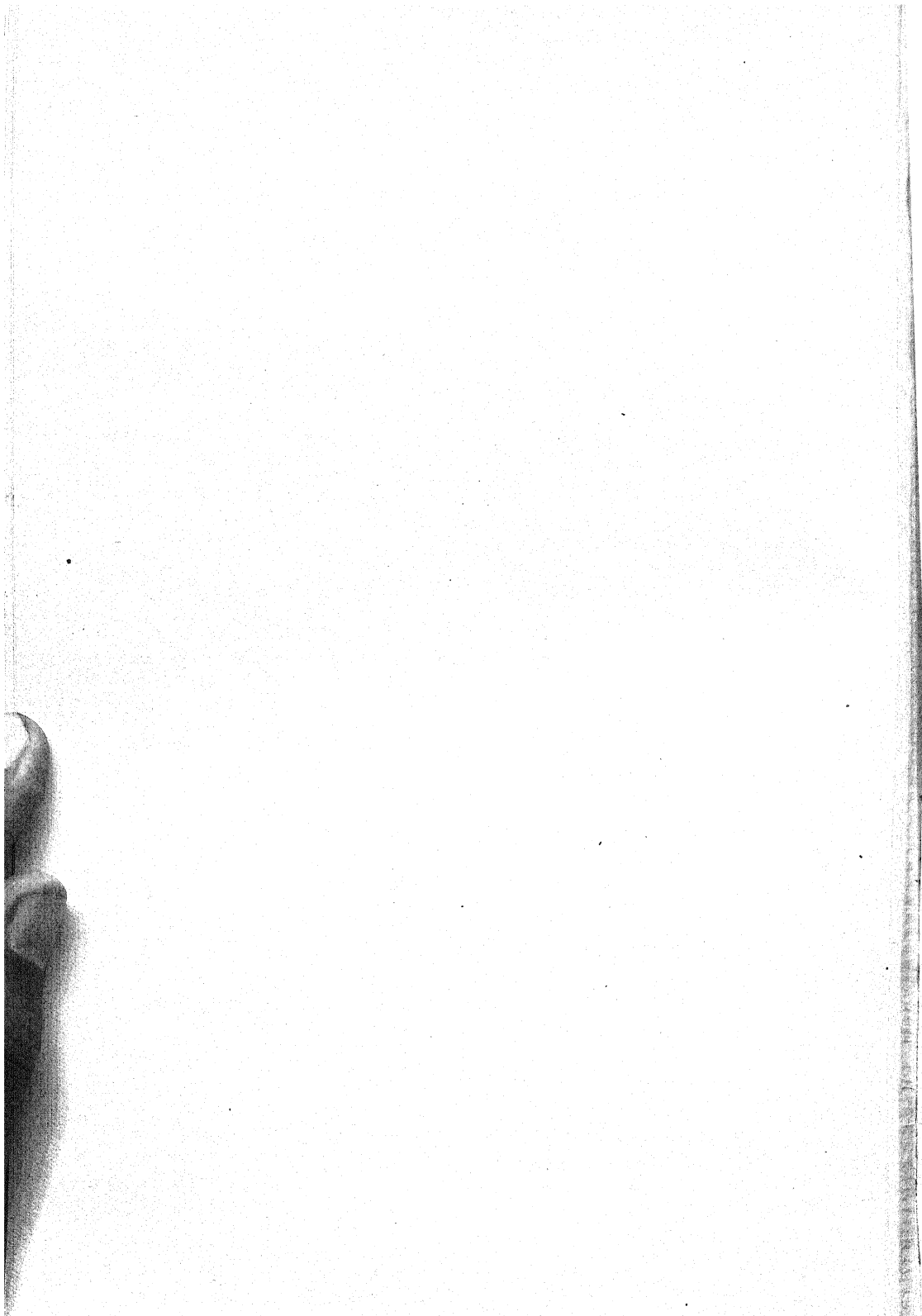
Hartnuth et imp.

of resin was but which received ammonium, reduced in dry concentration as containing less ammonium reduction per concentration application of ammonium.

MARCH I.—During months, the maintained a in 1, after 8 months second harvest was again reproduced from each of the five plots, and the plants were analyzed as before. of the data. In experiment I, magnitude of stems at the winter, was decreased on under leaves. Tre 6, or 60 % of the as ammonium which differed significantly as to dry weight percentage of tuber. The plant or 100 % of the yielded protein and rubber content.

EXPERIMENT I  
Experiment II parallel tomato and of V, in that growth stimulation of dry the ratio of ammonium nitrogen in the tuber. Both CLA pointed out the general type of the analysis the composition





# The 'Endoconidia' of *Thielavia basicola*, Zopf.

BY

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With Plate XXIII and one Figure in the Text.

## INTRODUCTION.

*THIELAVIA basicola*, Zopf, is a parasitic fungus well known to plant-pathologists. The ascigerous fruit which is referred to the Perisporiaceae has only once been obtained in pure cultures of the fungus,<sup>1</sup> and with this exception is connected with the other spore forms only by their association on the host plant and a doubtful tracing of continuity of mycelium.

The best known condition of the fungus is the black 'torula' or chlamydospore stage, which was described by Berkeley and Broome<sup>2</sup> as early as 1850.

The interesting 'endospores' escaped attention until a quarter of a century later, when the fungus was very thoroughly investigated by Zopf.<sup>3</sup>

It is with this stage of *Thielavia* that I propose to deal in the present paper.

Zopf describes these spores as being borne on short several-celled conidiophores and formed in acropetal succession. Their lateral walls then differentiate into two layers, of which the outer forms a sheath through which the conidia successively emerge. The cause of their extrusion is presumed to be a mucilaginous middle lamella which swells on access of water and so pushes out the spore.

<sup>1</sup> Peglion, V.: La moria delle piantine nei semenzai. Ricerche intorno ai mezzi de difesa. Staz. Sper. Agr. Ital., vol. xxxiii, fasc. 3, 1900, pp. 221-37.

<sup>2</sup> Berkeley, M. J., and Broome, C. E.: Notices of British Fungi: *Torula basicola*. Ann. and Mag. Nat. Hist., ser. 2, vol. v, 1850, p. 461.

<sup>3</sup> Zopf, W.: *Thielavia*, gen. nov. Perisporiacearum. Verhandl. Bot. Ver. Prov. Brandenburg, j. 18. Sitzungsber. 30. Juni 1876, pp. 101-5. Die Pilze, 1890, pp. 36, 81, 96, 113, Fig. 61. Ueber die Wurzelbräune der Lupinen, eine neue Pilzkrankheit. Ztschr. f. Pflanzenkrankh., Bd. i, No. 2, 1891, pp. 72-76.

There is some doubt whether at first, perhaps, Zopf regarded the conidia as produced endogenously; but later he discarded as superfluous the term 'Pseudosporangium' for the 'pistolenförmige Conidienbildungen', and apparently considered the peculiar formation of the spores to be correlated with a curious method of liberation.

Since this work much literature has accumulated around the subject, of which the greater part prior to 1909 is cited in the bibliography appended to Gilbert's<sup>1</sup> memoir.

This author, who treats of the morphology of the fungus with more than usual fullness, may be taken as representing the general opinion subsequent to Zopf's investigations. The 'endoconidiophore' consists of a tapering 'endoconidial cell' seated upon a few to several plump barrel-shaped cells. The 'endoconidia' are produced from the copious protoplasm within the terminal cell, which opens by a bursting or dissolution of the tip, and the conidia are slowly pushed out by the growth of the protoplasm in the swollen basal portion of the cell, new conidia being formed continuously in the rear of those being ejected. 'It is sometimes difficult to perceive that the conidia originate within the cell and are not formed by its direct septation.'

According to this interpretation the conidia are produced endogenously within the neck of a phial-shaped cell; and below the latest formed conidium is a naked surface of protoplasm.

Duggar<sup>2</sup> holds a slightly different view. The spores are formed by basipetal septation as short cylindrical cells within the branch. The tip of the latter is finally broken and the conidia are pushed out by osmotic force, the branch assuming the part of a spore-case.

Massee<sup>3</sup> describes the conidiophore as an upright septate branch becoming gradually narrowed above and remaining perfectly colourless. The apical portion becomes ruptured and the contents grow out through the torn apex as a chain of spores.

Professor V. H. Blackman suggested to me that this subject required a thorough investigation, and I am grateful to him for the laboratory facilities he placed at my disposal.

#### METHOD.

The differences between natural structure and artifact in fixed and stained preparations of minutiae in Fungi are frequently of so fine and nice a quality that their exact evaluation is very difficult. In the present

<sup>1</sup> Gilbert, W. W.: The Root-Rot of Tobacco caused by *Thielavia basicola*. U.S. Dept. Agric. Bur. Pl. Ind., 1909, Bull. No. 158.

<sup>2</sup> Duggar, B. M.: Fungous Diseases of Plants, 1909, p. 212, Fig. 83.

<sup>3</sup> Massee, G.: A Disease of Sweet Peas, Asters, and other Plants. Bull. Misc. Inform. Roy. Bot. Gard., Kew, 1912, pp. 44-52, Fig. 3. Mildews, Rusts, and Smuts. 1913, p. 50, Pl. XI.

investigation it was consequently felt very desirable to make as many observations as possible upon *Thielavia* in the living condition, and to use fixed material for confirmatory work.

Differential *intra vitam* staining by the prolonged action of very dilute aqueous solutions was largely employed. The fungus was grown principally upon banana or potato media, usually solidified by the addition of one to three per cent. agar; and that for preparation fixed *in situ* with either Bouin's fluid or weak Flemming's fluid. Alum haematoxylin with either acid fuchsin or Congo red was the stain giving the best results.

#### ORIGIN AND GROWTH OF THE CONIDIOPHORE.

The peculiarly shaped mother-cell or conidiophore arises from the middle region of a cell of the mycelium as a minute protrusion bounded by a very delicate and hyaline membrane. This point of origin, although perhaps not absolutely constant, contrasts sharply with that of an ordinary hyphal branch which is at the anterior end of the cell. Each cell contains a single nucleus which is minute, and appears either as an aggregation of deeply staining granules, or is well defined with granules often in immediate proximity.

An appreciable time after the inception of a conidiophore the nucleus of the parent cell divides, and one of the daughter-nuclei passes into the protrusion. The protoplasm in the latter is clear and has a higher refractive index than the vegetative cell contents. In its development it assumes a slightly curved finger-like form and soon is cut off by a transverse wall immediately above its base. The protoplasm becomes more dense, but rarely granular, and the nucleus occupies a position away from the tip of the cell.

When mature the conidiophore presents an exceedingly characteristic appearance (Pl. XXIII, Fig. 1), being slightly bulbous in its basal portion, and possessing an elongated, tapering, or almost linear apical region. The cytoplasm is often slightly alveolar or flocculent, occasionally minutely granular, and rarely slimy or homogeneous. Large vacuoles are usually present, which, particularly towards the upper region of the cell, not infrequently contain oil-globules. The nucleus lies in the basal portion of the cell.

#### FORMATION OF CONIDIA.

The nucleus in the conidiophore apparently divides in a mitotic manner, and one daughter-nucleus remains in the original position, whilst the other passes to the apical region of the cell (Pl. XXIII, Fig. 2). Here the protoplasm shows a barely perceptible increase in density, the vacuoles contract slightly in size and frequently contain a greater number of oil-globules. This apical region containing a nucleus slung in the protoplasmic

bridge between two vacuoles is now cut off from the conidiophore by a septum which grows inwards in the form of a ring or diaphragm, finally closing in the centre (Pl. XXIII, Figs. 3-4). The conidium almost invariably contains two vacuoles, thus presenting a very characteristic appearance (Pl. XXIII, Fig. 5 *a*). The oil-globules within the vacuoles may remain discrete, appearing as a cluster of grapes; or fuse to fill the entire vacuole so that it reacts as one large oil-globule; or be present as a fine emulsion within the vacuole. Rarely oil-globules may be found lying freely in the cytoplasm (Pl. XXIII, Fig. 4).

#### LIBERATION OF THE CONIDIA.

The liberation of the first conidium is brought about by a tangential splitting of its walls, which are thus differentiated into an outer closed sheath and an internal cell (Pl. XXIII, Figs. 5, 5 *a*, and 6). An exhaustive micro-chemical analysis of the conidiophore prior to this occurrence was carried out, but no layer corresponding to a middle lamella could be detected in the transverse wall, nor any incipient line of splitting in the lateral walls.

Almost simultaneously with its differentiation, the sheath is ruptured at or near its apex, and the enclosed conidium projected about one-quarter to one-third of its length beyond the open mouth of the sheath, appearing like a cork in a phial (Pl. XXIII, Fig. 7). Rarely the rounded tip of the sheath is torn away and may be observed fitting as a tiny cap on the end of the protruding conidium (Pl. XXIII, Fig. 7 *a*).

The protoplasm in the conidiophore is thus not naked at its apical surface, but bounded and separated from the sheath by a very hyaline and delicate transverse wall (Pl. XXIII, Fig. 7) whose thickness is one-half that of the normal cell membrane. This wall, which has been overlooked in all previous work, now becomes convex to the spore, and, owing to the growth of the conidiophore up through the sheath, pushes out the first conidium.

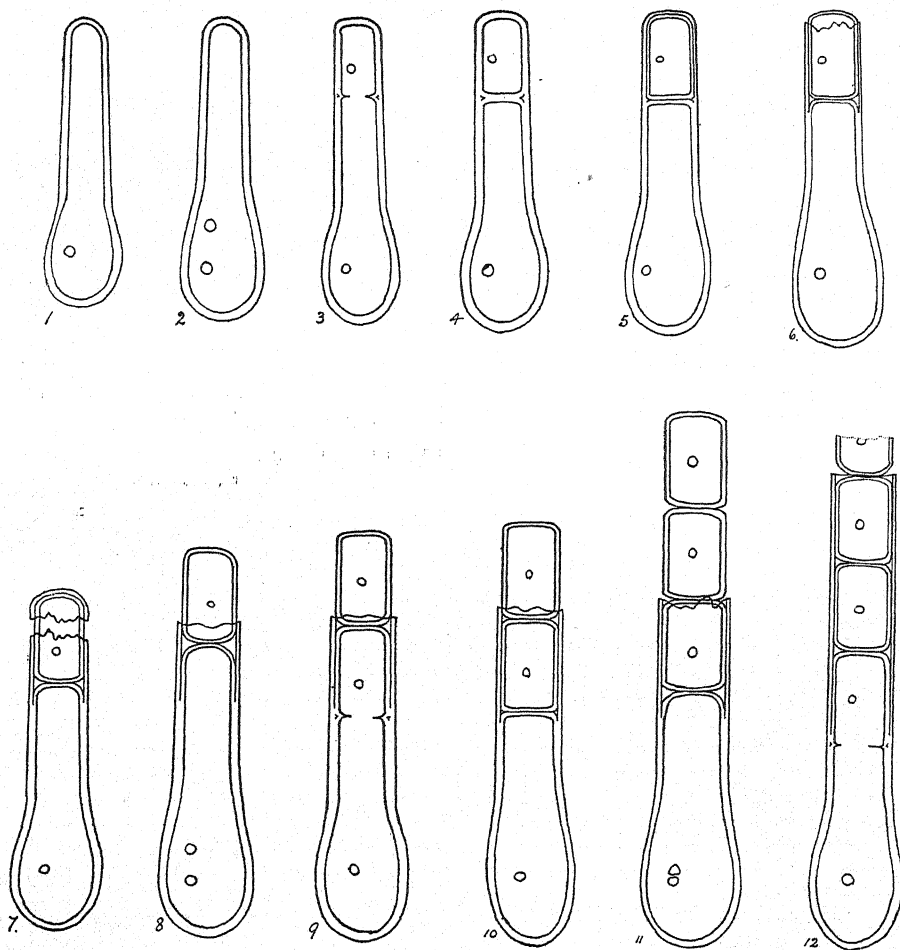
Meanwhile the nucleus in the conidiophore again divides, one daughter-nucleus passing to the apical region (Pl. XXIII, Figs. 5, 8, 9), which is then cut off as the second conidium, by a transverse wall formed in the manner already described. This wall is always immediately below the original position of the first wall, and when it is differentiated into two layers, the line of splitting being in the same tangential plane and meeting that between the conidium and the sheath, liberates the spore.

The latter is pushed out in the rear of the first by the development of the subsequent conidia, which are formed in like manner (Pl. XXIII, Figs. 5, 8, 9).

The conidia thus possess cell membranes which are only one-half the thickness of a normal cell-wall, this also being true of the sheath and elongating apical region of the conidiophore (Pl. XXIII, Fig. 7). Except

in early conidial formation the latter membrane is not easy to see, owing to the browning and increasing opacity of the sheath.

It will be noted that the development of each transverse wall adds a minute fraction to the length of the sheath. In consequence early conidial



TEXT-FIG. 1. Uninucleate conidiophore. 2. The nucleus divides and one daughter-nucleus passes to the upper end of the cell. 3. A transverse wall develops as an ingrowing diaphragm cutting off the upper region of the conidiophore. 4. The first conidium is delimited. 5. The wall of the conidium differentiates into two layers. 6 and 7. Rupture of the sheath and liberation of the conidium. 8 and 9. Formation of the second conidium. 10. Liberation of the second conidium. 11. Formation of the fourth conidium. 12. Late conidial formation at base of long sheath.

formation occurs at the base of a short sheath; whilst later it appears to take place some considerable distance within the neck region of the conidiophore, in reality at the base of a long sheath (Pl. XXIII, Figs. 8, 9). The latter therefore contains several spores at once, thus giving the appearance

of an active free cell formation occurring within an open tapering mother-cell or conidiophore.<sup>1</sup>

The process of conidial formation and liberation is diagrammatically represented in the Text-figure.

#### THE TRANSVERSE WALL IN CONIDIAL FORMATION.

Exact knowledge concerning the process of cell-division in Fungi is curiously limited, and the few studies which have been made yield discrepant results.

In the beak cells of *Basidiobolus ranarum*, Fairchild<sup>2</sup> has described the formation of a true cell-plate during the anaphases of division; whilst Raciborski<sup>3</sup> and Woycicki,<sup>4</sup> and more recently Olive,<sup>5</sup> maintain that the new wall grows in from the periphery as a constricting diaphragm, after the reconstitution of the nuclei. Olive<sup>6</sup> has also described a like process in *Empusa aphidis* and *E. sciaræ*. The gametes of *Sporodinia* and the conidia of *Erysiphe* are cut off in a similar manner, except that according to Harper<sup>7</sup> the apparent ingrowth here is simply a deep narrow furrow and not the growth inward of a ring of cell-wall substance. The wall in this case is deposited later between the two plasma membranes.

On the other hand, Baum<sup>8</sup> has described the laying down of a cell-plate during mitosis in *Coprinus ephemeroïdes* and *C. lagopus*; and this method has been confirmed by Maire<sup>9</sup> for *C. radiatus*.

<sup>1</sup> Under exceptional circumstances the conidiophores which normally produce thin-walled 'endoconidia' may give rise to chlamydo-spores. This first happened whilst repeating Peglion's experiments (loc. cit.) in a vain endeavour to obtain the ascigerous stage. Later it could be produced (though not with any constancy) by strikingly altering the conditions of the fungus, as for example from a state of desiccation to one of moisture and considerable warmth; or by treating the fungus with very dilute chemical solutions or mineral acids. Under such conditions of development the conidiophore usually grows right out through the sheath, and then behaves as a hypha of limited growth, forming chlamydo-spores in the normal way (see note on p. 8). The latter never resulted from the transformation of already formed hyaline conidia (compare *Thielaviopsis paradoxa* and *Sphaeronema adipo-sum*). Very often the formations were abnormal, the spores being thick-walled but irregular in shape. Not infrequently the conidiophores which had given rise to these thick-walled spores could be induced to return to their normal function by making the conditions natural again.

<sup>2</sup> Fairchild, D. G.: Ueber Kerntheilung und Befruchtung bei *Basidiobolus ranarum*, Eidam. Jahr. wiss. Bot. xxx 1897.

<sup>3</sup> Raciborski, M.: a. Mykologische Studien, I. Karyokinese bei *Basidiobolus ranarum*, Eidam. Bull. Internat. de l'Acad. des Sci. de Cracovie, 1896. β. Studya mykologiczne; Berichte der Akad. d. Wiss. zu Krakau, XIV. 2, 1899.

<sup>4</sup> Woycicki, Z.: Einige neue Beiträge zur Entwicklungsgeschichte von *Basidiobolus ranarum*, Eidam. Flora, 1893.

<sup>5</sup> Olive, E. W.: Cell and Nuclear Division in *Basidiobolus*. Ann. Mykol., vol. v, 1907.

<sup>6</sup> Olive, E. W.: a. Cytological Studies on the Entomophthoreae, I. The Morphology and Development of *Empusa*. β. Cytological Studies on the Entomophthoreae, II. Nuclear and Cell Division in *Empusa*. Bot. Gaz., vol. xli, 1906, I.

<sup>7</sup> Harper, R. A.: Cell Division in Sporangia and Asci. Ann. of Bot., vol. xiii, 1899.

<sup>8</sup> Baum: Über Zelltheilungen in Pilzhypen. Inaug. Diss. d. Universität Basel, 1900.

<sup>9</sup> Maire, R.: Rech. cytol. sur les Basidiomycètes. Bull. Soc. Myc. de France, 1902.

Faull,<sup>1</sup> working on *Laboulbenia chaetophora* and *L. gyrinidarum*, figures a delicate sheet of granules appearing across the diameter of the filament after the reconstitution of the nuclei. This becomes a definite cell-wall with a middle lamella.

In the conidiophore of *Thielavia* the transverse wall is formed by the ingrowth of a constricting membrane some considerable time after the reconstitution of the nuclei. The walls in the chlamydospores of this fungus are formed in like manner.

The phenomenon is apparently of cytoplasmic determination, and merely remotely or indirectly subject to nuclear control.

The formation of a transverse wall by a constricting ring-like growth may be brought about in two ways. The inner laminae of the parent wall may infold in the manner described for certain Algae;<sup>2</sup> or, as occurs in *Thielavia* and the cases described by Olive,<sup>3</sup> by a progressive deposition of new cell-wall substance upon a localized surface of the parent wall.

In its earliest stages the septum appears as a minutely granular, barely visible ring, becoming imperceptible at its ingrowing edge. With development its peripheral margin becomes more apparent, and a minute <-shaped mark may with difficulty be distinguished in the middle line of the parent wall opposite the diaphragm (Pl. XXIII, Figs. 3, 3 a, 8). On the completion of the septum the wall rapidly assumes normal thickness and appearance (Pl. XXIII, Fig. 4).

The >-shaped marking is in the line of the subsequent differentiation of sheath and inner wall and is probably a splitting apart of the laminae. The most careful microchemical analysis failed to reveal it as a substance.

In certain cases investigated by Olive<sup>4</sup> the new wall invariably grows inwards, constricting a vacuole; a protoplasmic bridge is later thrown across, and the completion of the membrane divides the vacuole into two portions. In *Thielavia* the new wall is invariably formed between vacuoles (Pl. XXIII, Figs. 3, 3 a).

In normal conidial development the formation of the transverse wall is complete; but occasionally in abnormal specimens a pore varying in diameter and admitting a wide protoplasmic strand is present (Pl. XXIII, Figs. 10, 10 a). In rare instances the growth of the septum is such that it bears striking resemblance to the lamellose plugs of *Codium* (Pl. XXIII, Figs. 11, 11 a).

It is interesting to note that in the transverse walls separating the chlamydospores, a single central pit is present. This, which is figured by

<sup>1</sup> Faull, J. H.: The Cytology of *Laboulbenia chaetophora* and *L. gyrinidarum*. Ann. of Bot., vol. xxvii, 1912.

<sup>2</sup> Brand, F.: Über Membran, Scheidewände und Gelenke der Algengattung *Cladophora*. Festschr. der Deutsch. Bot. Ges., 1908, where the literature is cited.

<sup>3</sup> Olive, E. W.: loc. cit., 1906.

<sup>4</sup> Olive, E. W.: loc. cit., 1906.



Zopf but overlooked by nearly all later observers, is always closed by a middle layer of cell-wall substance.<sup>1</sup> Rarely a pitted transverse wall, apparently affording protoplasmic continuity, could be distinguished in the vegetative hyphae.

#### DISCUSSION.

The development of 'endoconidia' is a process distributed sparingly but widely in the Fungi; and examples have been repeatedly described. In consequence the extensive literature on the subject is very scattered, and the synonymy of the forms has become greatly confused. Such species belonging to more than thirty genera are known, but it is probable that these may all be legitimately included in the following genera<sup>2</sup>—*Helotium*, *Sordaria*, *Phialea*, *Thielavia*, *Pyxidiophora*, *Sphaeronema*, *Thielaviopsis*, *Sporoschisma*, *Chalara*, *Cytosporella*, *Alternaria*, *Endoconidium*, *Hymenella*, *Bloxamia*.

A critical consideration and analysis of the published figures and descriptions, and in many cases of the Fungi themselves, showed an extraordinary similarity of form and, structure, size, and, where known, developmental details.

This likeness is so fundamental and complete as to nullify the discrepant accounts of the several authors, and, considering the extremely stereotyped character of the few methods of spore production known in Fungi, almost to preclude the doubt that possibly more than one process of development may be responsible.

<sup>1</sup> According to Zopf the chlamydospores are exogenous and their walls laid down simultaneously in the hypha (Simultane Scheidewandbildung, loc. cit., 1890): whilst Duggar states (loc. cit., p. 212) that their 'early stages of formation differ only in size from the endospores'; that is, they are endogenous and formed by basipetal septation. (Compare *Thielaviopsis paradoxa*, *Sphaeronema adiposum*.) My observations show that the spores are formed successively as thin-walled, barrel-shaped cells during the development of a hypha of strictly limited growth. They remain in this condition for some time and then gradually and simultaneously thicken their walls. It is after the walls have attained their mature thickness that the brown to black colouring matter is deposited. A slight 'lagging' in this latter process is apparent in those cells towards the apex of the chain. Frequently one to three or four cells at the base and rarely one or two at the apex remain thin-walled and colourless. Gilbert (loc. cit.) terms these the sterile segments; but I have frequently found them capable of immediate germination like the 'endoconidia'. This is interesting because the thick-walled chlamydospores will only germinate after a period of rest, in my experiments not less than ten weeks. This period may be very considerably shortened and even eliminated by subjecting the spores to a freezing process, to the action of dilute mineral acids, or of gastric juice. It is to be noted that in the case of gastric juice it is the acid and not the pepsine which renders premature development possible, the latter of itself having no effect on germination.

<sup>2</sup> See Additional Literature. Those Fungi which may be referred to the genera *Psiloniella*, *Glycophila*, *Sporendonema*, *Malbranchea*, and *Conioscypha* are not included in this list. In these cases what has been described as 'endoconidial formation' is better termed 'aplanospore formation', for each cell of the filament gives rise by rejuvenescence to a spore. Liberation occurs by the rupture of the mother-cells or the breaking down of the entire filament. The confusion has arisen by the frequent occurrence of this mode of spore formation in hyphae of limited growth, such that an appearance simulating an 'endoconidial cell' with a chain of spores is produced.

In only two or three cases has the mode of spore formation been developmentally observed, and then in but a cursory manner. On the other hand, in practically all, an endogenous origin of the spores by free cell-division within the 'endoconidial cell' has been assumed.

The more accurately these Fungi have been investigated the more irreconcilable are the facts with any hypothesis having a true and continuous endogeny as its basis, and the more exactly do they accord with the interpretation I have given of the process of spore formation in *Thielavia*. This latter is not one of endospory or endogeny, and the terms 'endoconidium', 'endoconidial cell', and 'endoconidiophore' are misnomers. The formation of conidia is a process of acrogenous abjunction ('acrogene Abgliederung'<sup>1</sup>), and it is only in the mechanism of their liberation that the peculiar character of these Fungi is seen.<sup>2</sup>

#### SUMMARY.

The conidia of *Thielavia basicola* are not endospores formed by free cell-division within an endoconidial cell. They are acrogenously abjoined from the conidiophore.

The first conidium is liberated by the differentiation of its walls into an inner wall and a sheath, and by the rupture of the latter at its apex.

The later conidia grow out through the sheath of the first, and are freed by the splitting of their basal walls.

The formation of the transverse walls is by the ingrowth of a ring of cell-wall substance which finally closes in the centre.

The process of conidial development seen in *Thielavia* is probably that of all 'endoconidia' in Fungi.

<sup>1</sup> de Bary, A.: Comp. Morph. and Biol. of the Fungi, &c., 1887, p. 61.

<sup>2</sup> It is interesting to note that neither de Bary (loc. cit.) nor Zalewski (Ueber Sporenabschnürung und Sporenabfallen bei den Pilzen, Flora, 1883) mentioned these forms, although certain of them were well known at the time.

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## EXPLANATION OF PLATE XXIII.

Illustrating Mr. Brierley's paper on *Thielavia*.

The figures were drawn with the aid of a Zeiss camera lucida. A Zeiss 2 mm. apochromatic 1.4 objective was used with a  $\times 12$  compensating ocular (Fig. 7), a  $\times 18$  comps. oc. (Figs. 3a, 5a, 10a, 11a), and a  $\times 6$  comps. oc. (remaining Figures).

Abbreviations used:—*c.* = conidiophore; *n.* = nucleus; *v.* = vacuole; *o.* = oil-globule; *p* = protoplasmic strand; *s.* = transverse wall; *sh.* = sheath; *sc.* = sheath cap; *sp'l.* = <-shaped marking outside transverse wall; *sp'l.* = line of differentiation of sheath and spore wall.

Figs. 1-4. Formation of the first conidium.

Fig. 1. Mature conidiophore.

Fig. 2. The nucleus divides and one daughter-nucleus (*n'*.) passes to the apex of the conidiophore. Specimen slightly plasmolysed.

Fig. 3. Formation of the transverse septum as an ingrowing ring of cell-wall substance.

Fig. 3a. Details of above more highly magnified.

Fig. 4. The completion of the transverse wall has cut off the first conidium.

Fig. 5. The walls of the conidium differentiate into two layers, the splitting being in the line of the <-shaped marking.

Fig. 5a. Details of above more highly magnified.

Fig. 6. A specimen strongly plasmolysed to show more clearly the differentiation of the walls of the conidium (reduced by one-half).

Fig. 7. The tip of the conidiophore shortly after the liberation of the first conidium.

Fig. 7a. The sheath, ruptured below the apex and its tip borne away on the conidium. It is interesting to note that in this case the conidiophore is the germ tube of a conidium.

Fig. 8. Late formation of conidia at the base of a long sheath.

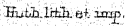
Fig. 9. A similar stage in which the conidia have been removed to show the sheath.

Fig. 10. Abnormal development of transverse wall, leaving a wide protoplasmic strand. Specimen much plasmolysed to show relations of walls.

Fig. 10a. Details of above more highly magnified.

Fig. 11. Abnormal development of transverse wall resembling lamellose plugs of *Codium*.

Fig. 11a. Details of above more highly magnified.



BRIERLEY — THIELAVIA BASICOLA.



## Studies in the Phylogeny of the Filicales.

### V. *Cheiropleuria bicuspis* (Bl.), Presl, and certain other related Ferns.

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With Plates XXIV and XXV and nineteen Figures in the Text.

THE Fern which now passes under the name of *Cheiropleuria bicuspis* (Bl.), Presl, was first noted by Blume in 1828 (Fil. Jav., p. 125, and Fl. Jav. ii. 175, Tab. LXVIII, B). He referred it to *Polypodium*, as *P. bicuspe*, Bl. It was subsequently described and figured by Sir William Hooker in the London Journal of Botany, vol. v, p. 193 (1846), with Plates VII and VIII. His specimens were collected by Thomas Lobb for Mr. Veitch, in the mountain tops of Java, and Sir William Hooker designated it as a splendid new species of an Acrostichoid Fern, ranking it with the genus *Gymnopteris*, Bernhardt, as *G. vespertilio*. But in 1849 it was constituted the sole representative of a new genus, *Cheiropleuria*, by Presl. It will be seen from what follows that it is properly ranked as the only known species of a substantive genus, and that the references to *Acrostichum* and *Polypodium* would only be possible in the older and most extended sense of those genera.

The Fern is rather widely distributed in the Malayan region. It is recorded from Malacca, Java, Sumatra, New Guinea, Borneo, the Philippines, South Annam, Formosa, and it even extends beyond the tropic to Oshima, the northernmost of the Loo Choo Islands (Christ, Geogr. der Farne, p. 164). It is moreover especially worthy of remark that it is commonly associated with *Dipteris conjugata*. This identity of distribution in Ferns which bear such a similarity as will be shown below, may probably be more than a mere coincidence.

After more than one unsuccessful attempt to obtain supplies of material for the examination of this peculiar Fern, which has never yet been submitted to anatomical and developmental study, Professor Bayley Balfour kindly passed on my wish for specimens to the Rajah of Sarawak.

This led to correspondence with the Director of the Museum at Sarawak, and with only very short delay I received from him a plentiful supply of specimens, both dry, and in spirit. They were obtained by him on a collecting trip to Mount Poi, Sarawak, in April, 1913. (See J. C. Moulton, B.Sc., F.R.G.S., Journ. Straits Branch R. A. Soc., No. 65, 1913.) From these it has been possible to ascertain all the essential facts of structure, though naturally certain developmental details must be left aside. In particular the gametophyte is still unknown. My hearty thanks are due to all those who helped in obtaining this material for observation, and especially to Mr. Moulton himself.

The best known of the published figures of the plant is that of Hooker, which is quoted by Diels (E. & P., i. 4, p. 337, Fig. 175). But more recently a photograph has been published by Christ (Geogr. der Farne, p. 18, Fig. 7), which gives a good idea of the appearance of the whole plant. There seems, however, to be some uncertainty as to the habit of this Fern, which probably arises from its being rather variable. The axis is elongated, and the internodes between the alternate leaves of various length (Fig. 1). It is densely clothed with silky yellow hairs, and bears many dark brown roots. Partly from the angles at which the petioles come off, and partly from the absence of soil from some of these rhizomes, it seems probable that some at least of them were climbers, others creeping on the ground. This would accord with the earlier descriptions. For Hooker (Sp. Fil. v, p. 272) mentions that in Java it is found 'on trees'. Diels (E. & P., i. 4, p. 336) describes it as 'epiphytic or terrestrial'; while van Rosenburgh (Malayan Ferns, 1909, p. 732), who probably has had the best opportunities for personal observation of it in nature, describes it as 'creeping or subscandent'. The material I have examined would accord best with this last description.

Dichotomous branching of the rhizome has not been observed. Lateral branches are, however, frequent; they arise on the abaxial face of the bases of certain leaves, but not of all (Pl. XXV, Fig. 2). The position is similar to that of the branches observed in *Lophosoria* and *Metaxya*, and a similar position 'on the hinder side of the stipes of each of the erect fronds' has been ascribed to like buds in *Platynerium alaicorne* (Higher Cryptogamia, p. 252). On the other hand, in *Matonia* (Seward, l.c., pp. 174 and 187, Fig. 6) the branching is dichotomous, as it is also in *Dipteris conjugata* (Seward, l.c., p. 494); and I find the same in *Dipteris Lobbiana*. Other modes of branching have not been observed in these Ferns. These data are in themselves interesting in their relation to the views of Velenovsky and of Schoute. The matter will be considered later, when the anatomical relations of the parts have been described.

The leaves are strongly dimorphic. The sterile have a firm leathery lamina borne on a thin wiry petiole of variable length, up to as much as a foot. The fertile leaves are taller, and bear a narrower lamina;



its under surface is covered by continuous soral masses on either side of the well-marked midrib. It was this feature which Hooker recognized as 'Acrostichoid'. There is a good deal of variety in the outline of the lamina. Hooker was aware of this; he describes the leaves as 'quite entire and tricostate, or sub-orbulate and deeply bicuspidate' (l. c., p. 271). He notes that specimens from the Loo Choo Islands were mostly with quite entire fronds; and these constituted the var. *b*, *integrifolia*, Eat. Both forms of leaf may be seen on the same plant (Christ, l. c., p. 18, Fig. 7), a point frequently met with among the Bornean specimens, in which also the entire leaves were in the majority. But they also showed examples of greater complexity of form, such as are represented in the photographs Figs. 4, 5. Comparison points towards the conclusion that the more complex outlines are liable to occur in the leaves of the more mature plants, but there is no constant evidence of an ontogenetic progression. Fig. 1 shows to the right a relatively weak plant, with all its leaves simple; to the left is a larger plant with two sterile and one fertile leaf, showing the difference in proportion of the two types. Of the sterile leaves one is ovate with a single cusp, the other is broadly two-lobed. On other plants it was not an uncommon thing to find three distal lobes united below into a broadly ovate lamina. Some plants, however, showed regularly the leaf-form depicted in Hooker's often quoted figure; this is shown in Fig. 3; but these were in a distinct minority in the specimens from Borneo. Hooker's figure, together with the specific name *bicuspe*, has in fact stereotyped much too strongly a form of leaf which is far from being general for the species. Other plants showed still more complex outlines, with irregular dichotomous branching. Thus in Fig. 4, one of the leaves is of the two-cusped type, but in the other both lobes have branched a second time. This condition is shown again in a more complete example in Fig. 5.

Comparing this last leaf with leaves of *Dipteris conjugata* it is at once evident that they conform to the same type, a conclusion which the venation also confirms. On the other hand, a comparison may also be made with the fertile type of leaf of *Platynerium*, and Fig. 6 shows one of these of *P. Hillii*, which corresponds in the number and relation of its dichotomies very closely with that of *Cheiropleuria* in Fig. 5. The chief difference lies in the continuance of the broad expansion downwards, so that there is no distinct petiole. But this is a condition which is still more pronounced in the nest-leaves of *Platynerium*, which have no petiole at all.

The essential features of the venation are already known from the drawings of Hooker, quoted by Diels (E. & P., i. 4, Fig. 175). But their arrangement at the base of the lamina is shown in Text-fig. 1, drawn from a leaf of *Cheiropleuria* made transparent by *eau de Favelle*, and mounted in Canada balsam. The main veins diverge in two groups right and left of the median line, while distally they show bifurcations. An examination of

their mutual relations at the base of the lamina shows that they are on a plan essentially similar to that in *Matonia* (compare Seward, l. c., pp. 175-6, and Fig. 1). It may then be concluded that the leaf in *Cheiropleuria* is a condensed and webbed example of the Matonioid type. When it is further remembered how the stages of progressive webbing are illustrated in the genus *Dipteris*, as well as in the related fossils *Clathropteris* and *Hausmannia*, it becomes clear that this is the true interpretation of the peculiar and variable forms of lamina seen in *Cheiropleuria*. (Com-



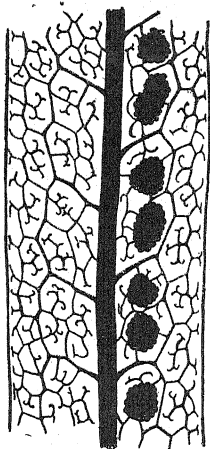
TEXT-FIG. 1. Trace of the vascular system at the base of the lamina of a large sterile leaf of *Cheiropleuria*, showing the pedate relation of the main veins, after the manner of *Matonia*. The smaller veins show the 'Venatio Anaxeti'.  $\times 4$ .

pare Seward, Phil. Trans. 194, Pl. 48, also Land Flora, p. 618. Also Seward, Fossil Plants, ii, pp. 386-94.)

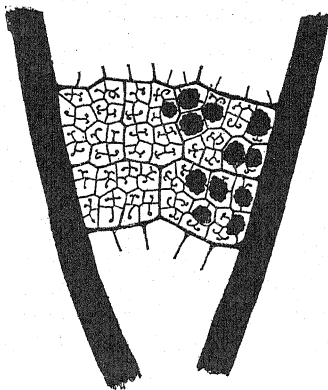
Between the main veins there is a reticulum, which is of the type described as 'Venatio Anaxeti' (Luerssen, Rab. Krypt.-Fl., iii, pp. 17-18, Fig. 22). This is the type also for *Dipteris conjugata* and *Lobbiana* (Text-figs. 2, 2 bis), and the same type, though in a more compact form, is seen in *Platycerium* (Text-figs. 13, 14).

The venation of the leaves of young plants of the Ferns above named would appear to present a promising line for further comparison. Fortunately, among the material of *Dipteris conjugata* collected by Professor Lang on the Malay Peninsula, some plants have been found which were

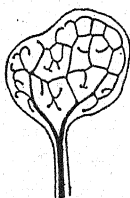
actually seedlings, or else stunted forms which retained or repeated the juvenile characters. These served for comparison with the youngest leaves available of *Cheiropleuria*, or the seedling leaves of *Platycerium*. Text-figs. 3, *a-d* are from *Dipteris conjugata*. They show that the reticulate



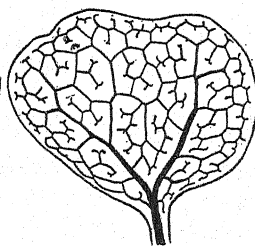
TEXT-FIG. 2. Part of a pinna of *Dipteris Lobbiana*, showing the venation, and its relation to the sori.  $\times 6$ .



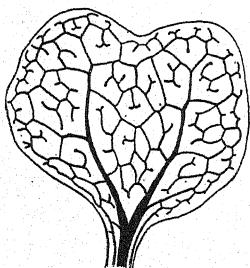
TEXT-FIG. 2 bis. Part of the lamina of *Dipteris conjugata*, including two of the main veins, and showing the venation between them, and its relation to the sori.  $\times 6$ .



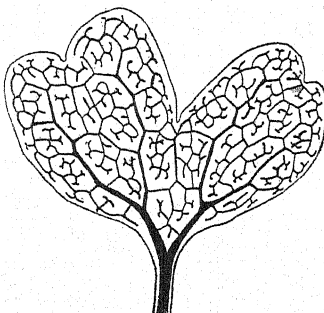
*a.*



*b.*



*c.*



*d.*

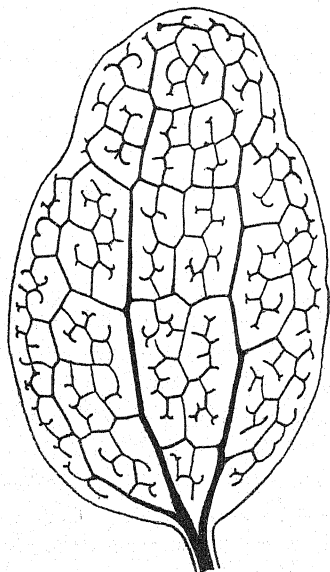
TEXT-FIGS. 3, *a-d*. Juvenile leaves of *Dipteris conjugata*, showing successive states of complexity of outline, and of venation.  $\times 6$ .

venation characteristic of the mature plant appears in the young leaves. The chief interest centres in the main veins. There is an apparent bifurcation in each at the upper end of the petiole; the two chief veins thus established may fork again (Figs. *b*, *d*), or their branchings may appear less regular. For purposes of comparison attention should be fixed upon the areolae which lie between the limbs of the first forking. In the smallest leaves (Figs. *a*, *c*) these areolae are less regularly defined, but in the more advanced (Figs. *b*, *d*) they are more definitely of square or polygonal outline, each with a venation within it ending in blind twigs. They present a very regular appearance in Fig. *d*. It may further be noted that as the forkings are repeated, in cases where the lateral lobes are strongly developed, there is a tendency towards a pedate development of the main venation. This is already recognizable in Fig. *d*, and it may become more marked in older leaves.

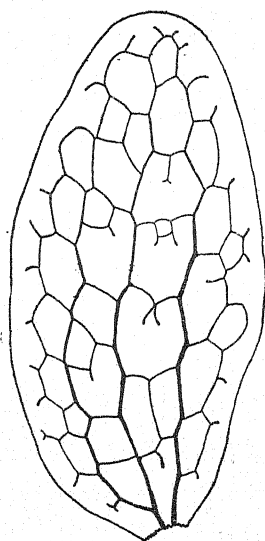
Comparing with these leaves the youngest leaf available of *Cheiropleuria* (Text-fig. 4), the outline of the lamina is here entire, there being no indication of bifurcation. But as in *D. conjugata* there is an apparent bifurcation of the main vein at the top of the petiole; the areolae between its shanks are of exactly the same type as in Fig. 3, *d*, while the main veins give off further branchings right and left, which correspond essentially to those seen in *Dipteris conjugata*. The similarity of the two is patent, allowing for the difference in outline of the leaves. A further step is to compare the young leaf of *Platycerium*. The young plants of *P. Veitchii* used were probably raised from vegetative budding. One of the youngest leaves (as yet undifferentiated as of the 'nest' or 'fertile' type) was removed. It is sessile, for which fact allowance must be made in the comparison. Its outline is very similar to that of the young lamina of *Cheiropleuria*, and its venation is obviously the same. Two main veins enter the base of the leaf, and behave in all essentials like those in *Cheiropleuria*. Similar areolae lie between them, but the venation within each of the areolae is of a simpler type (Text-fig. 5). This comparison of the young leaves shows that they all conform very closely to one type in their venation. It may be held as supporting the relationship of the three genera, which will be found to be strengthened by various other lines of similarity.

Comparison may be based upon the dermal appendages. Already Seward has noted the multicellular hairs on the rhizome of *Matonia* (l. c., p. 190), and has figured them (Pl. 19, Fig. 32). They appear to be all unbranched, and of the same type; and they form a dense covering. Each has a long setaceous indurated distal part, of two to ten or twelve cells, and a basal region of shorter thinner-walled cells, which suggest an intercalary growth at the base. Seward has also described for all the four species of *Dipteris* how the rhizome is covered by stiff brown scales, forming a dense felt (l. c., p. 493, Pl. 49, Figs. 29, 30, 34, 36). In the young state they are

simple hairs; but later the distal cells elongate and become brown and indurated, while the proximal cells remain short and thin-walled, and divide by longitudinal walls, so as to form a considerable solid base. All the hairs are here again unbranched, but the marginal cells project at their upper ends as blunt bosses, giving the margin a deeply sinuous outline. Thus their structure appears to be an advance upon the simple type of hair seen in *Matonia*. The difference is due to the longitudinal divisions at the base, but still the type of hair is essentially the same. In *Cheiropleuria* the type of hair conforms more nearly to that of *Matonia* than to that of *Dipteris*. Each is long and unbranched, and is always composed of



TEXT-FIG. 4. A juvenile leaf of *Cheiropleuria* showing the venation for comparison with *Dipteris conjugata*.  $\times 3$ .



TEXT-FIG. 5. A juvenile leaf of *Platycerium Veitchii*, for comparison with Text-Figs. 3 and 4.  $\times 3$ .

a simple filament of cells. But the induration is much less pronounced than that in either *Matonia* or *Dipteris*. Each may consist of as many as twenty or thirty cells. The type of hair seen in *Cheiropleuria* is thus probably as primitive as in any of these Ferns. On the other hand, in *Platycerium* the leaves, while young, and especially the fertile regions, are very efficiently covered by the well-known 'indumentum' of tufted hairs. Each hair consists of a stalk, with a multicellular distal star of about six rays. Clearly this is a more advanced state than that of any of the Ferns mentioned above.

A more complex type of dermal appendage is seen on the rhizome of *Platycerium*. In *P. alcinorne* the apical bud is densely covered with scales,

which are long and narrow, with a dark brown central rib. The distal end thins out to an acuminate apex, and may be terminated by a glandular cell. The midrib may be more than one layer of cells in thickness, and is composed of oblong cells with thickened brown walls, similar in their character to those forming the whole upper part of the hair in *Dipteris*. Laterally the appendage is flattened out into flaps of thin-walled cells, a single layer in thickness, while at the margins are borne fringes of hairs, each terminated by a glandular cell. At the base of the scale there remains for a time an active formative zone of delicate meristematic cells, a condition which compares with what is seen in *Matonia*, *Dipteris*, and *Cheiropleuria*. The whole scale is thus much more elaborate than in any of the preceding Ferns. But it may be held as a possible derivative of the type seen in *Dipteris*. A widening of the margins into thin lateral flaps, an elongation of the bluntly projecting marginal cells of *Dipteris* into multicellular hairs, and the appearance of terminal glandular cells upon them would convert the type of hair seen in *Dipteris* into the scale of the rhizome of *Platynerium*. And both are possible derivatives of the simple hair of *Matonia*, or of *Cheiropleuria*.

#### ANATOMY.

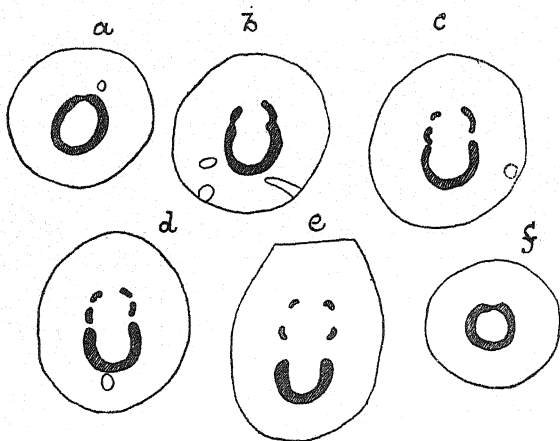
I am not aware of any detailed account having yet been given of the internal structure of *Cheiropleuria*. Christ (Farnkräuter, p. 128) makes a statement in his generic description which suggests a solenostelic structure; but none of my specimens confirms this. Sections of the rhizome, at whatever level or age of the specimen, show a protostelic structure. The stele is of considerable size, and in structure and in form it resembles closely that of the protostelic *Gleichenias*, such as *G. flabellata*, or *dichotoma* (Pl. XXIV, Figs. 8-12). It will then be unnecessary to describe it in detail. But it is to be noted that the protoxylem which enters from the leaf as a double strand (Fig. 11) merges at once on entry into a single strand (Figs. 10, 12), and can be traced only for a very short distance downwards below the point of entry. Accordingly, as a rule, only one such strand can be recognized in a given transverse section of the protostele. The xylem consists of rather wide tracheides, interspersed with parenchyma, which is more plentiful towards the centre of the stele. The band of phloem is characterized by narrow sieve-tubes, while the rather prominent proto-phloem is composed of specially small elements, the walls of which appear brown under safranin-haematoxylin. Outside this are about three layers of pericycle, and finally the endodermis. This delimits the stele from the broad peripheral cortex, of which the outermost layers are brown and sclerosed.

The leaf-traces are seen to originate from this protostele alternately right and left of the median line, on the upper side of the creeping or

ascending stele. First a group of small protoxylem tracheides appears some three or four layers below the outer limit of the xylem; opposite it the outer contour of the xylem projects as a rounded hump. Soon parenchyma cells aggregate internally to the protoxylem; the outer xylem then projects still more, and a loop of xylem with the protoxylem lying centrally within it is formed (Figs. 10, 12). This body of tissue continues to move outwards, some of the internally-lying tracheides following it, while laterally a constriction is formed, equally as a rule on both sides. As it deepens, the leaf-trace becomes gradually shut off from the stele by the intruding phloem and sheaths. On the separation of its xylem from that of the stele, it consists of an oval tract of tissue, enclosing within a complete ring of metaxylem a parenchymatous island, and on the peripheral limit of this lies the protoxylem, which has meanwhile divided into two groups (Fig. 11). Subsequently the trace becomes completely abstricted from the stele. It very soon opens out by separation of the metaxylem in a median plane, the lateral portions withdrawing, till the whole leaf-trace takes the form of a crescent, as in the cases of *Matonia* and *Dipteris conjugata*; the differences are that the leaf-trace is here narrower, and the protoxylem groups are only two in number, while the margins of the xylem are less strongly curved; there is also an enlargement of the xylem in the median plane. But this condition is only maintained for a very short distance. A median constriction soon appears (Fig. 13), and the xylem divides through the median enlargement above noted. The whole leaf-trace finally divides into two equal halves, each with its own protoxylem (Fig. 14), and in this state it passes out into the base of the petiole.

In the mere fact that the leaf-trace is at first undivided, *Cheiropteris* corresponds to *Gleichenia*, *Matonia*, and *Dipteris conjugata*. In its origination from a protostele it compares with all the simpler *Gleichenias*, and differs from *Matonia* and *Dipteris*, except in their seedling stage. It has been shown by Tansley and Miss Lulham (Ann. of Bot., vol. xix, p. 496) that the young seedling of *Matonia* has a protostelic axis. The same has been shown to be the case of *Dipteris Lobbiana*, by Miss de Bruyn (Ann. of Bot., vol. xxv, p. 761). The young plants of *D. conjugata* were also examined by her, but the material did not suffice for demonstration of the earliest phases. This deficiency has, however, been supplied by young plants of that species collected by Professor Lang, on the Malay Peninsula, and the section seen in Fig. 15 shows the protostele, from which a leaf-trace is just passing off. It may be taken then as usual for *Matonia* and *Dipteris* that the axis is at first protostelic, and that it passes through a 'Lindsaya stage' to solenostely of a more or less complicated type. Thus it is only with the youngest stages of these Ferns that the mature condition of *Cheiropleuria* can be compared. It has retained throughout its life the primitive state of *Gleichenia*.

But though *Gleichenia*, *Matonia*, and *Cheiropleuria* all agree in having an undivided leaf-trace, that is not a constant character of the genus *Dipteris*. It is so in *D. conjugata*, as has been shown by Seward (Phil. Trans., vol. 194, p. 498, Pl. 47, Fig. 4). But already Miss de Bruyn has observed that in the young plant of *D. Lobbiana* the leaf-trace comes off as two separate strands (l. c., p. 769, Pl. 57, Figs. 9–12). In mature plants it may be more complex still, and Text-figs. 6, *a–f* show by a succession of sections from below upwards how the trace arises. In *a*, the solenostele is about to open to form the foliar gap; *b* shows how, after opening, the margins are deflected outwards, while *c* shows how two strands have separated from the stele, and one of them has already divided;



TEXT-FIGS. 6, *a–f*. Successive transverse sections, from below upwards, showing the separation of the leaf-trace from the solenostele in *Dipteris Lobbiana*.  $\times 3$ .

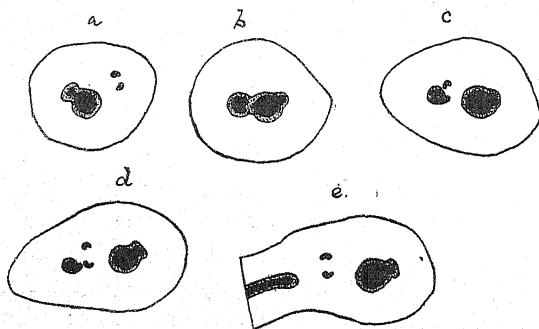
in *d* and *e*, both have divided, and the leaf-trace consists of four strands, in which state it passes out into the petiole: very shortly the solenostele again closes, as shown in *f*.

These facts are here adduced because they have an interesting relation to what is seen in *Cheiropleuria* on the one hand, and in *Platycerium* on the other. In the former the leaf-trace comes off, it is true, as a single strand; but it divides almost at once, in fact before it has traversed the cortex of the axis. In this respect it is in advance of the condition seen in *Gleichenia*, *Matonia*, and *Dipteris conjugata*. But *Dipteris Lobbiana* is again more advanced, since the trace originates as two separate strands, which divide again before the trace leaves the cortex of the axis. On the other hand, in the young plant of *Platycerium* Miss Allison has shown that the leaf-trace arises as two strands; but in the case of the mature leaf it is much more complex, consisting from the first, it may be, of a large number of separate strands (compare Text-fig. 9, below, p. 508). Thus in the matter of



complexity of the leaf-trace these Ferns may be held as forming a series, progressive from the simple condition seen in the simpler *Gleichenias*, and leading to the state seen in *Platyserium*. It will be seen later how far this runs parallel with seriation of them according to other characters.

It has been seen that branches are given off from the bases of many of the leaves, on the abaxial side (Fig. 1). In resolving the question of the nature of the branching, it is important to know the vascular connexions of the bud. This is shown for a given case of *Cheiropleuria* by the sections *a-e* of Text-fig. 7, which read from below upwards. The related leaf-trace originates from the axial stele in the normal way, and the protoxylem divides as usual as it enters the leaf-trace into two strands, which take the usual position. But the proportions of the leaf-trace strand are different from the normal. (Compare *a*, which is normal, with *b*, which bears a bud.) Its outline is

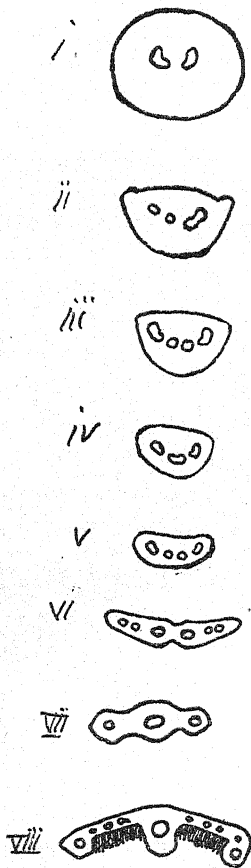


TEXT-FIG. 7. Transverse section of the rhizome of *Cheiropleuria*, to show the relations of the leaf-trace and the lateral bud to the stele of the axis. *a* shows the relation of two normal leaf-traces, where no bud is formed. *b-e* show successive sections from below upwards, in the case of a leaf-trace where a lateral bud is borne on the abaxial side of the leaf-trace.  $\times 3$ .

nearly circular (*b*), owing to an enlargement of the xylem on the abaxial side. Later the trace opens out, as in the normal leaf, on its adaxial face; then, first on the one side and then on the other, lateral hook-like processes are formed (*b*, *c*). These soon become detached as the two components of the leaf-trace, and as such enter the petiole. The large residuum of vascular tissue, lying in the median position with regard to the leaf, passes out directly as the stele of the lateral axis (*d*, *e*). The vascular relations are here essentially the same as those seen in *Lophosoria*, or *Metaxya*. As in those Ferns, so here in *Cheiropleuria*, the vascular connexions indicate that the lateral axis is an accessory appendage to the leaf. It would be difficult to see in them any evidence supporting the view that such branches are a result of some modified bifurcation. The conclusion will then be that dichotomous branching is in abeyance in *Cheiropleuria*, and that a formation of adventitious buds at the leaf-bases is the rule. This conclusion is, however, provisional, pending a searching examination of the ultimate

genesis of the bud, whether it be in relation to the apex of the shoot, or is really adventitious.

We have seen that in *Cheiropleuria* two strands of the leaf-trace enter the petiole: they maintain their identity for some distance (Text-fig. 8, i). The following details relate to a special case, and may be open to variation. About 3 inches above the base of the petiole the two strands divide, each into two (ii, iii); the four resulting strands pursue their course thus for about 2–3 inches further (iii), when those nearest the median line fuse (iv); this takes place about 2–3 inches from the base of the lamina. Later they again separate, the former arrangement of the four strands being resumed (v). The marginal strands then bifurcate (vi), to give six strands, the further course of which may be followed in the superficial views of the base of the lamina.



TEXT-FIGS. 8, i-vi. Successive transverse sections of the petiole of a sterile leaf of *Cheiropleuria*—i, at the base; ii, about 3 inches up; iii, 4 inches; iv, 6 inches; v, 8 inches; vi, 9 inches from base. Figs. vii, viii, are from a fertile leaf, at levels corresponding to Figs. v, vi of the sterile leaf.  $\times 8$ .

A comparison may be drawn with the petiole of *Dipteris Lobbiana*, as regards this behaviour of the strands of the petiole. It has been seen that the leaf-trace there also originates as two strands, which at once divide; so that four strands pass up the petiole (Text-fig. 6); thus the condition comes to be virtually the same as that seen in Text-fig. 8, iii. But at some point about two-thirds the distance up the petiole, the pairs of strands fuse again, and the two resulting strands have an obvious relation to the bifurcation of the lamina.

Such division of strands as this in the petiole of *Dipteris Lobbiana*, and in *Cheiropleuria*, and the subsequent fusion of the strands so as to close the gap formed, is only a special case of those arrangements commonly occurring in the petioles of Ferns, and giving the character of a divided leaf-trace. The point is most obvious in those cases where the leaf-trace is in other species of the genus an undivided strand, as in *Dipteris Lobbiana* and *Plagiogyria semicordata* (Ann. of Bot., xxiv, p. 431, Text-fig. 2). Such cases as these, occurring as they do without any relation to the pinnae or their pinna-traces, may be held to be 'perforations' of the petiolar supply, comparable in their nature to the 'perforations' so often seen in relatively advanced

Ferns in the axial stele.<sup>1</sup> Their occurrence in the Dipterid affinity has a special interest, in connexion with the comparisons with *Platynerium* to be instituted below.

In the case of the fertile leaf of *Cheiropleuria*, the outline of which is simple and narrow, the fertile region has as a rule a marked midrib, while two thickened ridges mark the margins. The extensive soral area covers the more or less extensive tracts within these, right and left of the midrib. A transverse section of the fertile lamina then shows in outline as in Text-fig. 8, viii. The difference between this and the sterile lamina is more apparent than real, as is seen if the vascular system is followed from below upwards. The petiole of a sporophyll shows at a middle level a condition as in (iii), and (iv) of the sterile; but above that level, instead of the fused median strand dividing again, as in the sterile petiole, it continues its fused course directly into the fertile lamina, where it forms the midrib (Text-fig. 8, vii, viii). A comparison of the result may be drawn between this condition of the fertile lamina and that of the leaf-segments in *Dipteris quinquefurcata*, or *Lobbiana* (Seward, l.c., Figs. 18, 24; or Land-Flora, Figs. 343-5). If we imagine the branched sporophyll of either of these species represented only by a single segment, it would have substantially the vascular structure of the fertile lamina of *Cheiropleuria*.

The venation of the distal end of the sporophyll is worthy of note, as in some degree harmonizing the difference between that of the sterile and fertile leaves. The soral areas frequently stop short of the extreme tip, which may then extend for some distance as a narrow beak. It may be traversed by a midrib with lateral reticulate branchings; or it may show two marginal ribs, with reticulations between them. Or, again, various other irregularities may be seen, including loops of the main veins quite comparable with those often found in *Dipteris*. All these facts appear to harmonize with the idea of the leaf of *Cheiropleuria* being a webbed and simplified example of the fundamental type seen in *Matonia* and *Dipteris*.

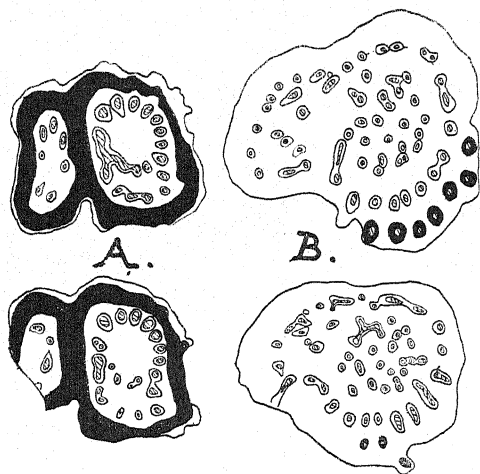
#### *Platynerium.*

As it will be necessary later to draw comparisons between *Cheiropleuria* and *Platynerium*, genera which have already been placed in near relation to one another by various writers, it will be convenient to introduce here certain facts relating especially to the vascular system of the latter genus. The similarity of the venation of the young leaves of *Platynerium* to that of *Cheiropleuria* and *Dipteris* has already been noted. Putting aside the remarkable dimorphism of the leaves in the genus, which is related to its epiphytic habit, the outline of the fertile leaves of *Platynerium* is often very closely conformable to that seen in the genera named. As an

<sup>1</sup> Compare Tansley, *The Filicinean Vascular System*, p. 65.

example, the leaf of *P. Hillii* shown in Fig. 6 might be very nearly matched by the more complex leaves of *Cheiropleuria*; at the same time it is impossible to miss the resemblance which they also show to so distant a type as *Ophioglossum palmatum* (compare Land-Flora, Fig. 238, p. 436). It is also to be noted that the leaves, both of *Platycerium* and of *Cheiropleuria* and of *Dipteris*, show the 'Venatio Anaxeti'. Accordingly, a comparison of the vascular system of their shoots should present points of interest.

Miss Allison has lately described the vascular system in the rhizome of certain species of *Platycerium* (New Phyt., vol. xii, p. 311, &c.). It was found that in *Pl. alcornae*, one of the less robust species, the vascular

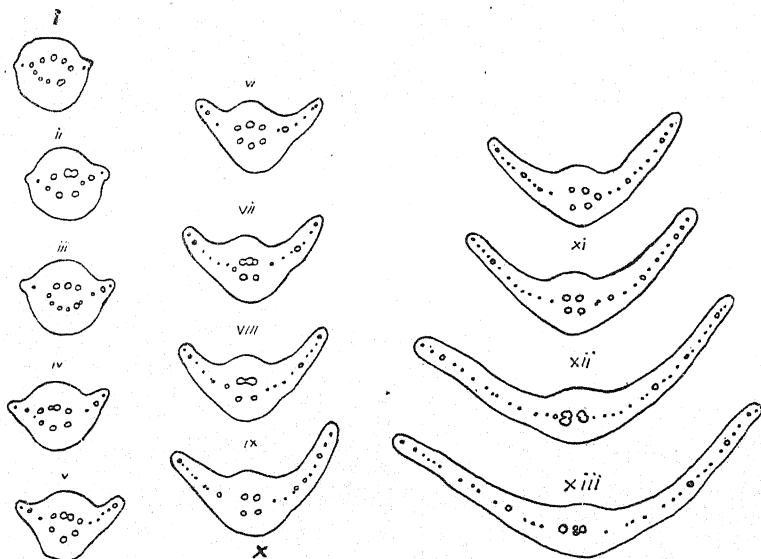


TEXT-FIG. 9. A. Sections of rhizome of *Platycerium alcornae*, showing relation of two leaf-traces to the ring of meristemes of the axis. B. similar sections from *Platycerium aethiopicum*, showing the greater complexity with numerous medullary strands. After Miss Allison, New Phytologist, 1914.

system of the axis is a simple dictyostele, but very highly perforated (Text-fig. 9, A); on the other hand, in *Pl. aethiopicum*, one of the most robust species, there is in addition a very complex medullary system (Text-fig. 9, B). As Miss Allison has pointed out, a comparison may be drawn with *Matonia* and *Dipteris* thus: 'Anatomically *Dipteris* is relatively simple; its simple solenostele is replaced by several concentric solenostelic cylinders in *Matonia*. In many other phyletic lines it may be seen how the solenostele becomes broken up into a dictyostele. It would be quite consistent with the structural facts here described, if we were to consider *Platycerium* with its complicated dictyostele as the dictyostelic type of a series of which *Dipteris* and *Matonia* are the solenostelic types' (l. c., p. 321). This seems to be a very reasonable interpretation of the facts so far as known; widespread 'perforation' would be an important factor in leading to the conditions described for *Platycerium*. Moreover, the fact that such perforations do occur in the petiole of *Dipteris Lobbiana* and of *Cheiropleuria* have a special

interest in this connexion. If the modifications there seen were extended into the solenosteles of the type of *Dipteris*, the state seen in the axis of *Pl. alcorni* would be the result; or into the axis of the type of *Matonia*, something like what is seen in *Pl. aethiopicum* would appear.

It has already been noted that in the young leaves of *Platycerium* the leaf-trace consists of only two strands. But in the mature state the trace comes off from the dictyostele of the axis as a number of distinct strands (*Pl. alcorni*), while in the more complex case where there is a medullary system in the axis, this also takes its share in the organization of the trace (*Pl. aethiopicum*) [New Phyt., vol. xii, p. 317]. The constitution and further



TEXT-FIG. 10, i-xiii. Successive transverse sections of the petiole of *Platycerium Hillii*, showing the disposition of the vascular strands. After the stage shown in xiii, the midrib settles down to three strong strands disposed in the plane of the leaf-expansion.  $\times 3$ .

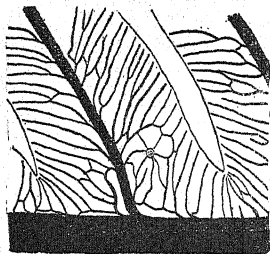
behaviour of the trace as it passes upwards into the leaf has been followed in the case of *Pl. Hillii*, a form nearly related to *Pl. alcorni*. The actual leaf examined was one of the fertile type, as shown in Fig. 6. Externally there is in the stalk an appearance as of a single central strand, or midrib, which throws off smaller veins right and left. But transverse sections show that the midrib is traversed not by one strand, but by a number of them, disposed in a circle (Text-fig. 10); and the condition is not unlike that seen in transverse sections of the leaf-stalk of *Ophioglossum palmatum* (Land Flora, p. 463, Fig. 259).

Starting from the base of the petiole, the strands are seen at first to be disposed in a simple circle (Text-fig. 10, i); their number varies, owing to splittings and fusions, which extend even across the adaxial face of the

circle (iii-iv, v-vi, vii-viii). From this circle come off at intervals strands of varying size, right and left. A comparison of Text-fig. 10, i-xiii, illustrates the method, and the way in which these spread, with further branchings and fusions, to form the reticulum of the flattened wings. As these wings expand upwards, the midrib gradually diminishes, and its strands simplify in number and arrangement, till with final fusions of abaxial and adaxial strands (xii) the circle is reduced to a single plane. After this the reticulum follows the course easily seen from the surface view. There is some similarity between this arrangement and what is seen in *Ophioglossum palmatum*, in the fact that in both there is a circle of strands, with fusions across the frontal face. But the correspondence does not extend into detail, and there is no correlative in *Ophioglossum* for the antero-posterior fusion of the strands of the circle itself seen in *Platycerium*. The facts point to an interesting homoplasmy in two quite distinct epiphytic types.

#### THE SPOROPHYLL IN *Matonia*, *Dipteris*, *Cheiropleuria*, AND *Platycerium*.

A comparative examination will now be made of the venation of the sporophylls in the Ferns in question, and its relation to their soral developments. It will be found that they form in a general sense a series, leading from simpler to more complex states; and that so far as this comparison is concerned, they form a rough sequence in the order in which they have been named in the above heading.



TEXT-FIG. 11. Portion of a pinna of *Matonia* to show the venation, and its relation to the sorus.  $\times 6$ .

The venation of the fertile leaf of *Matonia* is known from the observations of Seward (Phil. Trans., vol. 191, Series B, p. 175, Pl. 18, Fig. 23). The pinnules have a midrib, from which veins come off at a wide angle. They show frequent, but irregular anastomoses. But the most notable of these, as they are also the most regular, are those associated with the isolated sori. Each of the latter is seated at the centre of an areola formed by the lateral fusion of veins, while branches run radially in to the centre, where the receptacle is attached. At the base of the receptacle a few tracheides may be seen, but they do not extend conspicuously into it (Text-fig. 11).

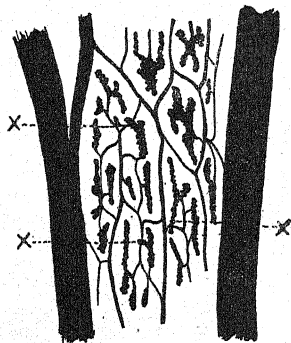
The condition of the fertile leaf of *Dipteris* is also known from the description given by Seward and Dale (Phil. Trans., Series B, vol. 194. See also Land Flora, Figs. 344-6). In *D. Lobbiana* the bifurcating lamina is narrower, and the sori are arranged in a more or less regular single row on each side of the midrib, as in *Gleichenia*. The venation is reticulate,

with two lines of areolae, and partially a third one, on either side of the midrib. Smaller twigs of vascular tissue extend into the areolae. Text-fig. 1 shows this, and the relation to it of the sori which remain on the one side of the drawing, but have been removed on the other. Each sorus is seated near to the centre of its areola, upon a vein connected with its margin. The vascular tissue does not show any characteristic extension into the receptacle, which in its position and in its vascular relations is similar to that in *Matonia*.

Much the same is the case with *Dipteris conjugata*, which is the broadly webbed species (compare Land Flora, Fig. 346). Through *D. quinquefurcata*, as explained elsewhere (Land Flora, p. 618), the transition is seen from the simple arrangement of sori on a narrow leaf to the webbed condition with very numerous sori scattered over its enlarged surface. But in *D. conjugata* the relation of the sori to the venation remains the same (Text-fig. 2); each is seated at the centre of its areola, above a branched vein which is connected with the boundary of its own areola. Thus whatever may be the differences of the sporangia in size, number, construction, or development, the relation of the sori to the venation is substantially uniform, so far as observation goes, in the genus *Dipteris*.

But when we pass to *Cheiropleuria*, while the relation to the venation is still essentially as in *Dipteris*, it will be seen that an extension occurs which is important in facilitating a comparison further with *Platyserium*. The fertile leaf differs from the sterile in being long and narrow, and upright in position. Its venation is the same as that of the sterile leaf in essentials, but upon a contracted plan; so that the areolae are much less numerous. If an examination be made of the thinner area of the fertile leaf, right and left of the midrib, which the soral area seems to cover entirely, and the venation be traced, it will appear as in Text-fig. 12. Clearly the method of venation is as in the fertile leaf of *Dipteris*, as would indeed have been expected from its similarity in the sterile leaves, old and young. But the important difference appears, that whereas in *Dipteris* the terminal twigs do not appear distended as wider storage tracheides, this is a marked feature in *Cheiropleuria*. Here the endings form considerable tracts of xylem, composed of tracheides with a high proportion of breadth to length, and these tracts may themselves be considerably elongated, while they extend so markedly towards the lower surface of the leaf, on which the sporangia and paraphyses are seated, that the distal tracheides lie very closely below the sporangial stalks. Further, it may occasionally be seen (as at 'x' in Text-fig. 12) that these receptacular extensions are not limited to a single areola, but pass in a lower plane than the limiting vein into the next areola, actually crossing the course of the vein limiting the areola, but in a lower plane. This is not a very marked or frequent feature in *Cheiro-*

*pleuria*, though it may happen repeatedly in near juxtaposition, as is seen in the case represented in Text-fig. 12. But it provides the explanation of that state of the fertile leaves of *Platycerium*, which, though described long ago by Hofmeister, and by Mettenius, has not yet been brought into line with other observations in Ferns. Mettenius, in his *Filices Horti Lipsiensis* (p. 26, Pl. IV, Figs. 1-3), described the double vascular system which occurs in *Platycerium*. He showed how in the fertile region of this Fern fine branches spring from the vascular network of the normal leaf; these anastomose to a superficial network immediately within the lower surface: its meshes are elongated, and always much narrower than those of the sterile region, and only seldom give off free twigs. It is only this superficial



TEXT-FIG. 12. Part of lamina of *Cheiropleuria* with two large veins, showing the relation of the smaller veins to the patches of receptacular storage-xylem. x, x, are points where the receptacular storage-xylem, in a lower plane, has crossed the limits of an areola of the venation which lies in a higher plane.  $\times 6$ .

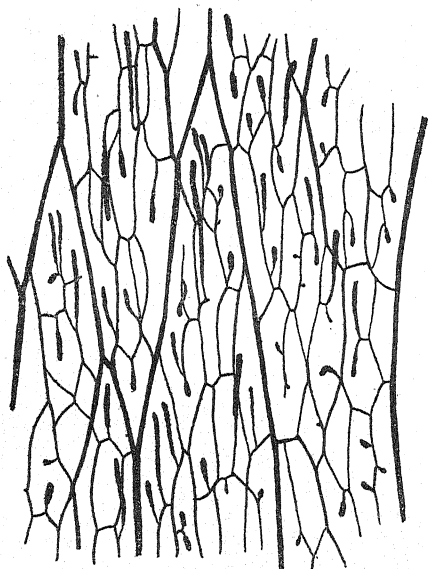
network which bears the sporangia. Hofmeister, in his *Higher Cryptogamia* (Engl. edn., p. 252), described a similar condition in the circular or reniform nest-leaves of *P. alcinorne*. 'Their vascular bundles lie, not in one, but in two planes parallel to the surface of the frond. These bundles form two many-meshed nets, one close under the upper side, the other immediately above the lower side of the frond; the two networks are united in many places by frequent ramifications, which pass through the mass of the frond in a transverse direction.' In my *Studies on Spore-producing Members*, No. IV (Phil. Trans., Series B, vol. 192, p. 86) the main results of these observers are confirmed as regards the fertile leaves, by examination of those of *P. alcinorne*, Desv. The double vascular system was thus known to exist in the leaves,

and especially in the fertile areas of *Platycerium*. Also that the soral areas were not indiscriminately spread over the leaf-surface, but restricted to more or less parallel lines. This comes out most clearly in those cases where the fertile area is less prolific, and the leaf has the appearance of being only half fertile. Occasionally in these the sori appear quite isolated, and relatively short. It is such leaves as these which give a ready basis for comparison with what has just been described for *Cheiropleuria*.

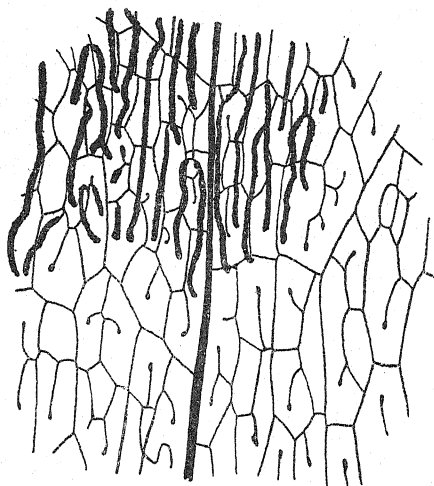
A semi-fertile area of *Platycerium aethiopicum* of this character is shown in Text-fig. 13. The venation is of the *Dipteris*-type, as it is in *Platycerium* generally. The free twigs of the venation are elongated, though more greatly than they are usually in *Cheiropleuria*, and they bore downwards so that their distal ends approach the lower surface of the leaf. Often each is restricted to its own areola; but in not a few cases, on arriving



at that lower level in the mesophyll of the leaf, it extends past the limiting vein of its own areola, thus crossing into the next. This is what has been seen to happen occasionally in *Cheiropleuria*; but it is a much more pronounced feature here in the partially fertile leaf of *Platyserium*. Still more is it so in the normal fertile area, as is shown in Fig. 14. Here the receptacles are much more elongated, and occasionally branched, and follow a distinctly parallel course. Their connexion with the main venation is as



TEXT-FIG. 13. Part of a lamina of *Platyserium aethiopicum*, which is only half-fertile, i. e. with the sori isolated and small: for comparison with Fig. 12.  $\times 3$ .



TEXT-FIG. 14. A similar drawing from a fertile leaf of *Platyserium angolense*. The position of the sori is upon the distal ends of blind veins; these elongate and enlarge as in *Cheiropleuria*, and pass to a lower level in the mesophyll: they extend frequently across the limits of the areola of the main venation which lies in a higher plane.  $\times 3$ .

before; but once they have passed into the lower plane in the thick and fleshy leaf, they may extend to great length, and cross not one only, but several areolae, and branch in their course. And thus there is constituted that second vascular system of the fertile area recognized by Mettenius. The appearance in transverse section is shown in Pl. XXV, Fig. 16, in *P. Willinkii*, from which it is seen that the two vascular systems extend in quite different planes, and that the receptacular strands are in close relation to the insertion of the sporangia.

While we may thus feel a special interest in this elaborate extension of the receptacle, and in the prolongation of its tracheidal system to form a sort of vascular system of its own, it may be recalled that such a development does not stand alone. In various types of Ferns the vascular system of the receptacle is liable to spread beyond the restricted area of the primitive sorus. It has been seen how in *Saccoloma* the marginal sori are isolated;

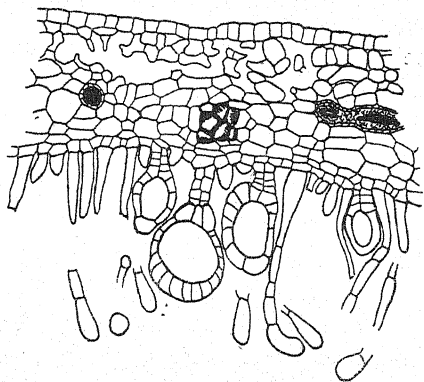
but in *Lindsaya* they are laterally confluent; the vascular system of their receptacles is there linked by commissures, so as to form a continuous strand. The origin of this state is by lateral spreading of the isolated sori of the *Saccoloma*-type (Studies, III, Figs. 20, 21). Similarly in the Pterideae, as was long ago pointed out by Prantl (Engler's Bot. Jahrb. iii, p. 403, &c.), while *Pellaea* has the sori separate, at the distal ends of veins, in *Pteris* they are confluent. The veins are linked by vascular commissures, which are really lateral extensions of the vascular supply of the receptacle. Again, in Studies, IV, a strong probability has been advanced that the primitive condition from which the Blechnoid Ferns have been derived was that with isolated sori, such as are seen in *Matteuccia intermedia*. By lateral extension of their receptacles, and especially of their vascular systems, the fusion-sori typical of the Blechnoids was produced. It has also been shown (Ann. of Bot., vol. xxviii, Pl. XXIX, Fig. 20f), that occasionally the tracheidal system of the receptacle in these Ferns may extend separately from the conducting venation of the leaf, and pursue a course of its own in a slightly different (lower) plane. This, though slight in extent in the case quoted, is similar in essential character to the larger receptacular extensions described for *Platycerium*. And thus it appears that in a number of distinct phyla of Ferns, the receptacle, and very markedly the vascular system of the receptacle, is liable to extension. This may result in a mere linking together of isolated sori, as suggested by comparison of *Matteuccia* with *Blechnum*, or of *Pellaea* with *Pteris*; or it may lead to an extensive vascular system with elongated sori attached, as in *Cheiropleuria* and *Platycerium*. However peculiar the latter case may itself appear, the above comparisons indicate that it cannot be held to stand absolutely alone. It may be held to be a specially pronounced example of a widespread phenomenon, viz., the extension of the individual sorus.

The sori of the series of Ferns at present under discussion show a forward progression in several features. The most important are, (i) the number of the sporangia, (ii) the order and time of their appearance, and (iii) the extent of the receptacle which bears them. The number of the sporangia in the sorus of *Gleichenia* is small, though it rises in certain species, which are on that and other grounds held to be in advance of the rest (Studies, II, Ann. of Bot., xxvi, pp. 274-5). In *Matonia* also the number is only from six to nine, and they are individually large, and are produced simultaneously. In *Dipteris* the number may be larger, and the individual sporangia small. In point of order and time of appearance of the sporangia, the simpler species correspond to the type of *Gleichenia*, or *Matonia*; this is so for *D. Lobbiana*, where the sori are disposed as in these Ferns in a simple series on either side of the mid-rib, and the sporangia arise simultaneously. But in *D. conjugata*, with its broad-webbed leaf, the sori are scattered over the enlarged surface, and

show a mixed condition, as regards the order of appearance of the sporangia (Land Flora, p. 621). But this is not actually an 'Acrostichoid' condition, for the sori are still circumscribed, and the receptacles are quite distinct, as is shown in Text-fig. 2; and individual sporangia do not occur as a rule upon the areas between the receptacles. In *Cheiropleuria* the venation is the same as that in *D. conjugata*; but the sori are not circumscribed. They are merged into a continuous mass of sporangia and paraphyses, which covers the whole surface, the identity of the sori being completely lost. The sporangia are borne indiscriminately over the whole surface, and not only at points above the vascular receptacles. Further, sporangia of very various ages are found in juxtaposition, giving a pronounced 'mixed' character to the whole mass (Text-fig. 15). Thus *Cheiropleuria* is fully 'Acrostichoid', and non-soral, and shows a marked advance upon the other types as regards these characters.

*Platycerium*, on the other hand, is not truly 'Acrostichoid', though it has often been assumed to be so. It has already been shown that the fertile patches of this genus are not continuous, but that the sporangia are disposed along definite lines, which are really extended sori (Phil. Trans., B, vol. 192, p. 86). They lie above those vascular strands, which constitute in each case an elongated receptacle, and form collectively the lower vascular reticulum (see Text-fig. 15). The sporangia are disposed upon these in two more or less definite rows, one on either side of these elongated receptacles. Moreover, they originate for the most part, if not wholly, simultaneously. Thus the fertile patches of *Platycerium* are really composed of closely placed aggregates of greatly extended sori, themselves essentially of the type of the Simplicis, so far as the origin of their sporangia is concerned.

It thus appears from the facts adduced that the soral condition of the Ferns under discussion is referable in origin to a primitive state seen typically in *Gleichenia*, with a single row of circumscribed sori on either side of the relative midrib; and with few sporangia produced simultaneously in the sorus. It has been shown (Studies, II, Ann. of Bot., xxvi, p. 275) that in *G. pectinata*, by crowding of the sorus, it had become mechanically inefficient, since there is not sufficient room for median dehiscence of its sporangia. From this relief might be found (1) by increasing the length of the sporangial stalk, (2) by adopting lateral dehiscence, (3) by extending



TEXT-FIG. 15. Transverse section of part of a fertile lamina of *Cheiropleuria*.  $\times 50$ .

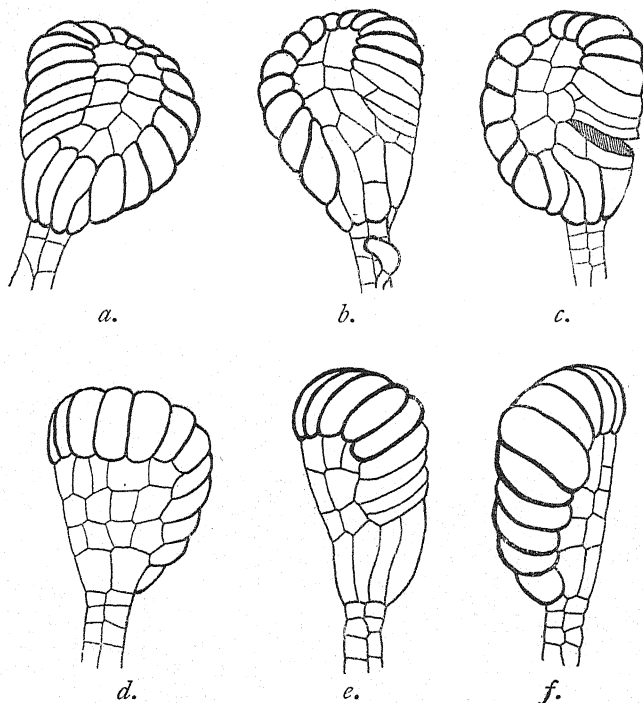
the area of the sorus, or (4) by elongating the receptacle. *Gleichenia* did not adopt any of these devices, but other Ferns did, and have succeeded. The *Matonia-Dipteris-Cheiropleuria* Series has adopted the three first named, but not the last, for none of them have developed as Gradate types.

*Matonia* itself retained the circumscribed sorus, but adopted a lateral dehiscence of its short-stalked, but large and few sporangia. In *Dipteris* the number of the laterally dehiscent sporangia is larger, their size smaller, their stalks longer, and the area of the sorus larger, especially as seen in *D. Lobbiana*, and *quinquefurcata*. In *D. conjugata* the less size of the sorus is balanced against their much greater number spread over the webbed lamina. In *Cheiropleuria* the laterally dehiscent sporangia are still longer-stalked, and the confluent sori are spread continuously over the leaf-surface, giving a fully 'Acrostichoid' character. In *Platycerium*, however, the sori are merely elongated, not confluent, the large number of the sporangia being accommodated by the extension of the area of their elongated receptacles. The latter types appear to have diverged far from the simple Gleichenioid source, which would hardly be recognized in them were it not for the intermediate states which some of them show, and also the comparisons which may be based on other characters.

#### THE SPORANGIA.

The sporangia of *Matonia* are well known, and need no fresh description (see Seward, Phil. Trans., vol. 191, p. 171; Studies, IV, Phil. Trans., B, vol. 129, Pl. 4, Figs. 59–62, and Land Flora, p. 565). But those of *Dipteris* are less fully investigated. They have been figured and described by Seward (Phil. Trans., vol. cxciv, Pl. 48, Figs. 11–16) and by Miss Armour (New Phyt., 1907). But these analyses were not exhaustive, and their development has never been fully traced. It will be seen that their segmentation gives an important line of comparison with *Cheiropleuria*. The sporangia of *Dipteris Lobbiana* are shown from their 'peripheral' side in Text-fig. 16, *a*, *b*, and it is apparent that the annulus is a complete ring of cells, though the induration of those opposite the stalk is incompletely carried out. A comparison may be made with a similar view of the sporangium of *Gleichenia lineata* (= *Gl. dichotoma*) (Land Flora, p. 555, Fig. 310), which shows similarity of form and of position of the annulus; but there the induration is complete, and the dehiscence distal and median. In *Dipteris Lobbiana* the dehiscence is obliquely lateral and, as the Text-figs. 16, *a*, *b* show, either right or left of the median line. The stomium is not well defined, a character shown also in *Matonia* and *Gleichenia*. The stalk is short, and shows two rows of cells, as seen from the peripheral side. Fig. *c* shows a similar view of a sporangium already dehiscent. In Fig. *d* is a view from the 'central' side, but with the annulus hidden on the dehiscent margin. Again the stalk appears as two rows of cells. Since

this appearance is shown from both sides, it follows that the stalk has the unusual composition of four rows of cells, a point definitely demonstrated by transverse sections. Text-figs. 16, *e* and *f*, show sporangia seen obliquely from the side, the first from the side of the stomium, the other from the completely indurated side. From these various views the structure of the sporangium will be fully realized. The similarity of form, and in certain features of the annulus, to that of *G. lineata* is obvious; but though the annulus remains oblique, as in that Fern, its induration is incomplete,

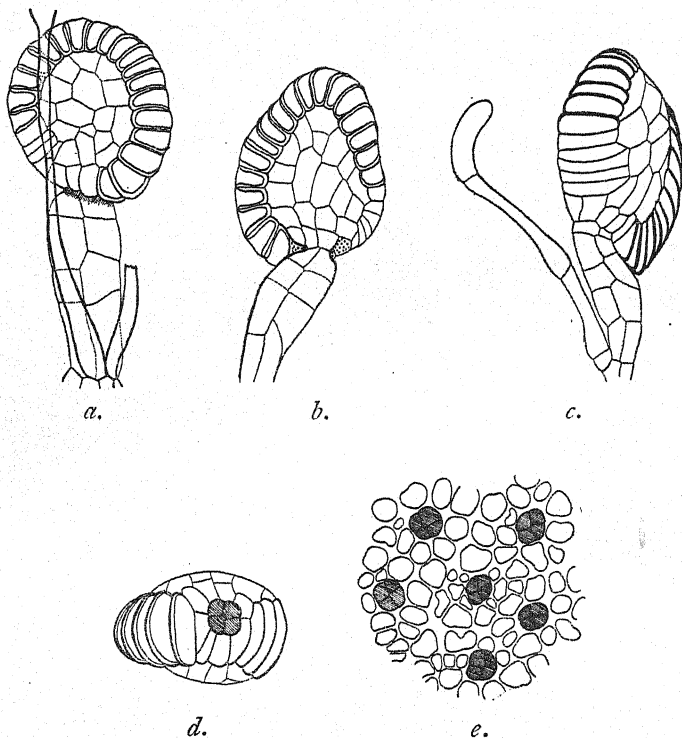


TEXT-FIGS. 16, *a-f*. Various aspects of the sporangia of *Dipteris Lobbiana*.  
For description see Text.  $\times 50$ .

while the stomium has swung into a lateral position; and the stalk is of a less complex construction.

The sporangium of *Cheiropleuria* is larger, and longer-stalked than that of *Dipteris*, but the general type of it is the same, a very distinctive point of similarity being the four-rowed structure of the stalk. This is clearly shown by tangential sections of the soral area, in which the sporangial stalks appear densely surrounded by paraphyses (Text-fig. 17, *e*). Seen from the 'peripheral' side the sporangium appears as in Text-fig. 17, *a*, associated with paraphyses, which are longer than itself until maturity is reached. The stalk shows two rows of cells, which become slightly constricted below

the insertion of the head. The latter has a continuous series of cells of the annulus, but the induration is not continued past the stalk; moreover, the cells opposite the stalk, which are thus thin-walled, are less regular in outline than the rest. Laterally there is a stomium which is in a position unusually near to the insertion of the stalk, that is, distinctly below the equator of the sporangial head. It is more regularly constructed here than in *Dipteris*, being usually composed of four cells; this point is best seen in Text-fig. 17, *c*, which represents an oblique view, with the stomium facing

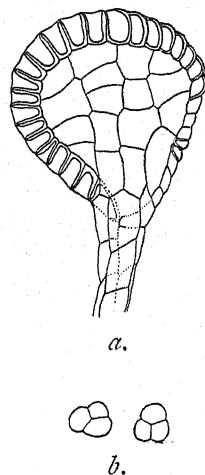


TEXT-FIGS. 17, *a-e*. Various aspects of the sporangia of *Cheiropleuria*. For description see text.  $\times 80$ .

the eye. The 'central' side of the sporangium is shown in Text-fig. 17, *b*, where the constriction of the stalk just below the head is very marked. Here also the relation of the continuous annulus to the stalk is seen, but it is best understood from Text-fig. 17, *d*, which shows from below the insertion of the stalk (shaded), and the way in which the series of cells of the annulus extends, though with modified form and without full induration, continuously past it. The details of the sporangia are, however, not rigidly constant; thus the number of the cells of the annulus may vary; such numbers as thirty-six, thirty, and twenty-six have been counted.

Moreover, it is not always equally clear that the annulus is continuous past the stalk. Such differences are natural, and are even to be anticipated in cases like the present, where, *ex hypothesi*, the sporangium has undergone modification from a more primitive type.

For comparison with the above, the sporangia of *Platycerium* have been examined, and a typical example is shown in Text-fig. 18, *a*, for *P. aethiopicum*. One point of difference is in the stalk, which here consists of only three rows of cells, as is clearly indicated by transverse sections (Text-fig. 18, *b*). The form of the sporangial head is more pear-shaped, and the annulus less clearly oblique, while it retains the lateral position of the stomium distinctly below the equatorial line. Opposite the insertion of the stalk the annulus is drawn down into an acute angle, in accordance with the pear-like form of the sporangium, and it is almost interrupted, but not actually so; for the rather elongated cells on either side do, as a matter of fact, retain contact, as is shown by the dotted lines in Text-fig. 18, *a*. Comparing this sporangium with that of *Dipteris* or *Cheiropleuria*, it is seen to be of a more advanced type, as shown by the thinner stalk, the more vertical annulus, and the almost completed interruption of it at the insertion of the stalk.



TEXT-FIG. 18. *a*, sporangium of *Platycerium aethiopicum* as seen from the side. *b*, transverse sections of its stalk.  $\times 80$ .

#### DEVELOPMENT OF THE SPORANGIUM.

Unfortunately the material of *Cheiropleuria* did not, in age or in preservation, suffice for tracing the development of the sporangium fully. But the essential features have been observed, and a comparison may be drawn with the very similar development in *Dipteris conjugata*. The general features of the fertile leaf have been described above. If sections of the fertile region be examined, the flattened expansion is found to be relatively thick, and it has a spongy texture of the mesophyll towards the upper surface. The mesophyll is traversed by two distinct types of vascular tissue; first, the normal venation, lying in a median plane, and with the small and compact strands clearly circumscribed by parenchymatous sheaths. Two of these strands are shown, right and left, in Text-fig. 15. Secondly, there are found lying between these, and closer to the lower surface, more lax strands composed of larger tracheides of the storage type, and without parenchymatous sheaths. These are the receptacular endings of veins, which, as seen in surface view, are liable to be greatly enlarged and elongated (Text-fig. 12).

The lower surface of the leaf is covered by the dense sorus, which is not limited to any position above the veins, but is spread uniformly over the whole area. It consists of (a) very numerous paraphyses, simple unbranched hairs, each terminating in a glandular cell; (b) isolated sporangia disposed with no definite regularity among them. The relations of these are shown in transverse section in Text-fig. 17, *e*, and in vertical section in Text-fig. 15. The sporangia are seen in the latter to be sometimes seated over the vascular tracts; but they appear to be equally common in the areas between them. Further, as they are not disposed in any order of age, the soral areas are of the 'mixed' type. Thus there is a definite 'Acrostichoid' condition of the sorus in *Cheiropleuria*.

While young, the sporangia are efficiently covered by the paraphyses, which are taller than they. But as maturity approaches, the sporangial stalk elongates, so that the sporangial head comes to be level with their ends, and can discharge its spores freely, while those which are younger are still protected below. This arrangement is closely parallel with that of the *asci* in any of the Discomycetous Fungi.

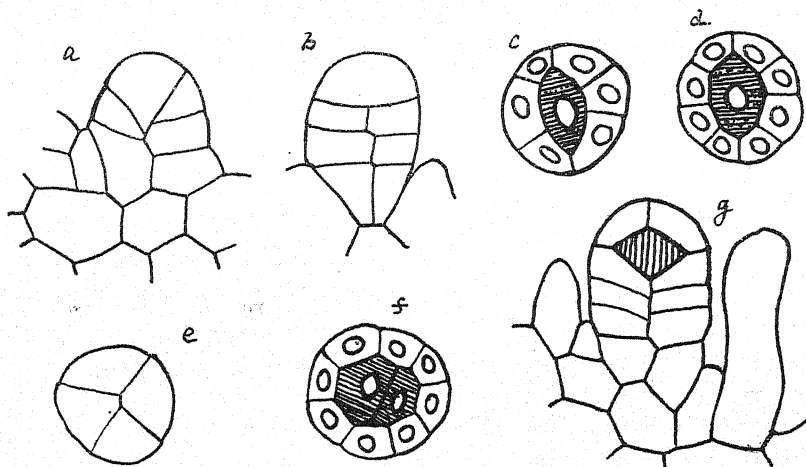
The sporangium originates from a single superficial cell. In a large number which have been examined, the segmentation appears to show a regular cleavage of the segments in two opposite rows. Naturally, those selected for drawing show these right and left, for thus they present the most definite appearance. The first of such segment-walls cuts obliquely down to the base of the square mother-cell (Fig. 17, *a*). The number of the cleavages does not appear to be constant (compare Fig. 17, *b-e*). After the number of the cells in each row, either by primary cleavage or by subdivision, has reached four to six, the wedge-shaped cell which occupies the apex undergoes periclinal division (*d*), which is followed by further segmentations parallel to the last, giving rise to the tapetum, and the central sporogenous cell (*f*). Later development follows the course usual in Leptosporangiate sporangia. Meanwhile, subdivision of the two rows of segments of the stalk by walls in the plane of the drawings has given rise to the four rows of cells of the stalk, as seen in the later stages (Text-fig. 17, *d*).

The cleavages thus described are so peculiar and exceptional among Leptosporangiate Ferns that it was thought well to compare them with those seen in *Dipteris*, in which a like structure of the sporangial stalk has been seen. Here again the storage tracheides of the receptacle lie closely below the surface where the sporangia are borne (Fig. 18, *a*). The sporangia arise from single cells, which may here have an oblique base, and the first oblique segment-wall impinges upon that oblique face; thus the type of sporangium is from the first less robust than that of *Cheiropleuria*. Alternate segments in two rows then follow, as in *Cheiropleuria*. In order to check the fact of this alternate segmentation, sporangia were examined



also from the side facing one of the rows of segments, instead of taking them in profile. Such a view is shown in Fig. 18, *b*, the appearance of which is consistent with the segmentation by two alternate rows of cleavages. Transverse sections also bear out the correctness of the conclusion (Fig. 18, *c*). Miss Armour has already shown (New Phytologist, 1907) that in these sori the sporangia are not all of the same age, and Fig. 18, *a* and *c*, confirm this. Some are found to show only two cells, corresponding to the two rows of segments; others have undergone subdivision of the segments, and show as the result the four rows of the mature stalk.

*It is clear from these observations that 'Dipteris' and 'Cheiropleuria' provide a new and distinct type of segmentation of the Fern-Sporangium. The similarity which these genera show in other features, when taken to-*



TEXT-FIGS. 19, *a-g*. Segmentation of young sporangium of *Metaxya*. *a, b* are two lateral views, at right angles to one another: *c, d* show the sporangial head in transverse section: *e* is a transverse section of the sporangial stalk; *f*, a transverse section of an older sporangial head: *g* shows a sporangium of the age of *c, d*, seen laterally.  $\times 300$ .

gether with their similarity in this rare feature, forms a convincing body of evidence of their real affinity.

The only other type of Fern which has been described as having constantly four rows of cells of the sporangial stalk is *Metaxya* (Ann. of Bot., xxvii, p. 447, Pl. XXXII, Fig. 8). The detail of segmentation of the young sporangium, as viewed from above, was not looked into when the plant was being investigated; but it will be seen at once that Figs. 5, 6, on the plate quoted, show numerous segmentations, as seen from the side, which would accord with a two-sided segmentation. Fresh observations were therefore made with the results shown in Text-figs. 19, *a-g*. The drawings *a, b* show sporangia in which the cap-cell has not yet been formed, from points of view at right angles to one another; in *a*, the

wedge-shaped cell from which segments have been cut off right and left, is seen on edge; in *b* it is seen from the side, and the segments themselves have divided into equal halves, thus giving the four-rowed structure of the stalk, as seen in *e* when cut transversely. In *c*, *d*, which show views from above, the two-sided segmentation is clearly demonstrated; while in *g* the cap-cell has been cut off, and has already divided. Thus the segmentation of the sporangium in *Metaxya* accords with that of *Cheiropleuria* and *Dipteris*. It has been shown that *Metaxya* on comparative grounds may be held as related to *Lophosoria*, and be regarded as a Gleichenioid derivative. *Dipteris* and *Cheiropleuria* have probably a similar relation, and all the three genera correspond in this uncommon feature of the two-rowed segmentation of the sporangial primordium.

Lastly, for purposes of comparison, a section of the fertile region of the leaf of *Platyserium* is represented in Pl. XXV, Fig. 16. Here the leaf is thicker than in *Cheiropleuria*, and the sori are localized above the receptacular strands. It is clear from the section that the latter run here in a plane much nearer to the lower surface than the strands of the regular venation. In *Cheiropleuria* (Text-fig. 15) the difference of level between the receptacular strand and the true venation is only slight; but here, in Fig. 16, strands of the two systems are seen directly superposed, and several layers of cells intervene between them. This is in accordance with what has been stated above (p. 512), and with the drawing of Mettenius (Filices, Hort. Lips., Pl. 4, Figs. 1-3). It may also be noted that the three sporangia shown in Fig. 16 are at approximately the same stage of development, though that which is median is slightly in advance of the others. This is characteristic for *Platyserium*. The origin of the sporangia is almost simultaneous, but slight differences of time may be observed in them.

#### COMPARISONS AND CONCLUSIONS.

It will be evident from this detailed description of *Cheiropleuria*, incomplete as it is, and entirely deficient as regards the gametophyte, that the genus is one which has a special comparative interest; and that the interest lies, not only in the location of the Fern in its own probable phyletic position, but also in the demonstration that it brings of the divergences from strict parallelism, in respect of the various characters which are used as criteria in such comparisons. It is only when such divergences are fully taken into account that a correct valuation can be set upon the method of phyletic study, based upon such comparisons, and of the conclusions which follow from them.

The first question will be as to the probable phyletic position of *Cheiropleuria*, and especially its connexions *downwards* in the scale. Then may follow the question of its probable relations *upwards*, with forms still

more advanced than itself. There can now be little question of the relations of *Cheiropleuria* downwards. Its nearest affinity is with *Dipteris*, and less closely with *Matonia*. This is shown by the similarity of form of the shoot, and of the dermal appendages. The form of the lamina, and especially of those variants of it which have more than two cusps, and the venation also, link it clearly with *Dipteris*. The steps towards a webbed character of the lamina seen in *D. Lobbiana*, *quinquefurcata*, and *conjugata* lead, in a manner which cannot be overlooked, to the more complete integration of the lamina as it is seen in the one-cusped type so frequent in *Cheiropleuria*. This conclusion is also borne out by comparison of the outline and venation of the leaves of young plants of *Dipteris*.

In venation, the types of *Gleichenia*, *Matonia*, *Dipteris*, and *Cheiropleuria* form an interesting sequence. In *Gleichenia* the venation is open throughout, thus showing a state which is usually held to be primitive. In *Matonia* there are occasional vein-fusions, especially in relation to the sorus. But in the narrow-leaved species of *Dipteris* such fusions are so frequent as to form a reticulum, while in the webbed species *D. conjugata*, the type is that of 'Venatio Anaxeti'. This venation is characteristic also of *Cheiropleuria*, as it was also of the fossil genera, *Dictyophyllum*, *Clathropteris*, and *Hausmannia* (see Seward, Fossil Plants, vol. ii, p. 380, &c.), which have been referred to the Dipteridinae. Thus, as regards the venation, *Gleichenia* corresponds to the type prevalent in the Palaeozoic Period; *Matonia* takes a middle position, and the Dipteridinae show a venation characteristic of Mesozoic or later periods. *Cheiropleuria* is, as regards this character, as recent as any of them, notwithstanding the primitive structure seen in its axis.

The Matonioid Ferns, including the Dipterids, have been habitually regarded as *Gleichenioid* derivatives (Land Flora, pp. 622, 623). But hitherto the anatomical conditions have not materially helped this comparison, for in the mature state both *Matonia* and *Dipteris* have advanced solenosteles, while *Gleichenia* shows in most of its species a protostelic state. It is here that *Cheiropleuria* comes in as a synthetic link. The comparison of its protostelic axis with that of *Gleichenia* (Figs. 8-12) is very striking. Similarly its leaf-trace comes off at first as an undivided strand, though its early division into two indicates a state advanced beyond that of any *Gleichenia*, or indeed of *Matonia*. But it shows some similarity to what is seen in *Dipteris Lobbiana*, which is even more advanced in the division of its leaf-trace; for it there arises as two or even four distinct strands (Text-fig. 6).

As regards the protostelic state of *Cheiropleuria*, it may be noted that a similar protostely has been found in the young plant of all of the related Ferns which have been examined. Thus *Cheiropleuria* maintains to maturity that primitive anatomical state which others have departed from early.

A minor character which also indicates a primitive state is that the dermal appendages are all hairs. Flattenedramenta are absent, though they may be found even in some *Gleichenias*. As compared with the hairs of *Matonia* and *Dipteris*, those of *Cheiropleuria* are simpler, and suggest that in this feature it is the most primitive of them all.

The comparisons given in detail above have shown that, as regards the sorus, *Cheiropleuria* is an advanced type. This is seen from its 'Acrostichoid' soral areas, and from the 'mixed' condition of the sporangia. But the comparison instituted through examination of the vascular supply shows that the state seen in *Cheiropleuria* is relatively primitive compared with that seen in *Dipteris* and *Matonia*, though closely resembling that seen in *Gleichenia*. The sori of all of these are superficial, and *Dipteris*, with its variable soral conditions, gives the connecting clue between the simple state in *Gleichenia* and *Matonia*, and the 'Acrostichoid' state of *Cheiropleuria*. The arrangement in *D. Lobbiana* is clearly conformable to that of *Matonia* and *Gleichenia*. The advance seen in *D. quinquefurcata* leads, with the addition of the webbing of the frond, to that seen in *D. conjugata*, with its enlarged leaf-surface. Upon this the numerous sori retain their identity; but in *Cheiropleuria* that identity is lost, and the result is seen in the large continuous fertile patches. Such progression from a state with discrete sori to the merged, 'Acrostichoid' state finds its parallel in several other phyletic lines in Ferns. (Compare *Annals of Botany*, 1914, p. 427.)

In their sporangial characters *Cheiropleuria* and *Dipteris* are closely alike. The most striking point of that similarity lies in the four-rowed stalk, which so readily results from the peculiar cleavage of the sporangial primordium by alternate segments ranging in two rows. The resemblance of the genera in a character so remarkable is the strongest possible evidence of correctness of the comparisons based upon other features.

Taking all the characters together, the conclusion seems fully justified that *Cheiropleuria* is a Fern of Matonioid-Dipterid affinity. That while it has retained a primitive type of dermal appendages, and a vascular structure of its axis such as is seen in the presumable Gleichenioid ancestry, it has adopted a type of leaf and of sorus which are seen in the relatively advanced species of *Dipteris*, but has carried these features to a still more advanced state than is seen in any member of that genus. It is a striking example of the absence of strict parallelism of advance in the several criteria of comparison. But at the same time the incongruity of its characters marks it out as one of the most interesting synthetic types to be found among living Ferns.

Turning now to the relation of *Cheiropleuria* upwards in the scale, that is to forms which, though probably related, show collectively characters of still further advance than *Cheiropleuria* itself does, these are found

especially in the curiously modified epiphytes of the genus *Platynerium*. In *Cheiropleuria* a scandent tendency has been seen, though it does not appear to have led to any definitely climbing or epiphytic state. But *Platynerium* has its shoot strongly modified in relation to epiphytic life, as shown particularly in its peculiar leaves. Nevertheless, externally a similarity to *Dipteris* and *Cheiropleuria* may be traced, not only in the outline of the erect or pendent leaves, with their regular bifurcation, but also in the venation; and this may be traced even in the highly modified nest-leaves. Perhaps it is in the newly-described species, *P. Sumbawense* n. sp. Christ (Warburg, Monsunia, i, 1900), that the similarity between the fertile leaves and those of *Dipteris* is the most marked. For there the narrow lamina is repeatedly furcate, so that the lacineae may number twenty on a single frond, and all are soriferous; this is reminiscent of the simpler species of *Dipteris*, such as *D. Lobbiana*. The comparison already made of the juvenile leaves of *Cheiropleuria* with those of young plants of *Dipteris* and of *Platynerium* supports the similarity still further, and it is apparent also in the mature leaves of *D. conjugata*, of *Cheiropleuria*, and even in some measure in the nest-leaves of *Platynerium*.

But the anatomical comparison of the axis shows marked divergence of character in *Platynerium* from what is seen in *Cheiropleuria* or *Dipteris*. As Mettenius has shown, and Tansley has quoted in his Lectures on the Filicinean Vascular System (p. 62), the axis of *P. alpicorne* is highly dictyostelic, and perforated. There is, as a result, an almost simple ring of meristeles, while the leaf-trace, with certain complications at its origin, comes off *ab initio* as a number of detached strands (Text-fig. 9, A). Miss Allison (New Phyt., 1913, p. 311) has recently published a description of this with figures, and has also described the vascular system for *P. aethiopicum*. In the latter the structure is still more complex, for the leaf-trace consists of still more numerous strands, while a large number of medullary strands are present at the centre of the axis (Text-fig. 9, B). A comparison of the structure of these species with the states seen in *Matonia* and *Dipteris* suggests that the relatively simple ring of meristeles seen in *P. alpicorne* finds its correlative in the simple solenostele of *Dipteris*; while the more complex system in *P. aethiopicum*, with the numerous medullary strands in addition, finds its correlative in the polycycl of *Matonia*. Imagine these genera with their solenosteles profusely 'perforated', and something very like the stem structure of the species of *Platynerium* would be the result. Clearly *Platynerium*, on such an interpretation, takes anatomically a more advanced position than *Dipteris*, or *Matonia*, and a still more decidedly advanced position than *Cheiropleuria*.<sup>1</sup>

<sup>1</sup> See The Genus *Alcicornium* of Gaudichaud, by L. Underwood. Bull. Torrey Club, vol. xxxii, 1906, p. 587, &c. Underwood desires to retain the name *Alcicornium*, Gaud., in place of *Platynerium*, Desv. But I follow Christensen's Index in retaining *Platynerium* as the generic name.

But in respect of the venation in the fertile region of the sporophyll, *Cheiropleuria* is in closer relation to *Platycterium*. It has been shown above how the receptacular strand in *Cheiropleuria* may pass, in a lower plane, out of its own vascular areola, thus initiating an independent course of its own. The same may be found in *Platycterium*, though here it is on a larger scale; and it leads to the formation of that second vascular system described by Mettenius, which spreads in a lower plane, between the main system of the leaf and the sori themselves. This condition is so exceptional among Ferns that the degree of correspondence demonstrated between *Cheiropleuria* and *Platycterium*, in this respect, gains thereby additional weight. It may therefore be held that the points of similarity seen in their leaves go far to outweigh the differences of stelar structure in the stem between these two genera.

In its dermal appendages—whether the flattened scales of its rhizome, or the stellate hairs which cover the young sori—*Platycterium* shows an advanced condition. But the sorus itself, though the receptacles are greatly elongated, are still not typically 'Acrostichoid', as in *Cheiropleuria*; each, though prolonged, maintains its individuality, while the sporangia originate almost simultaneously. The sporangia themselves have the usual three-rowed stalk, and the annulus is more definitely interrupted than in *Dipteris* or *Cheiropleuria*. Thus sorally *Platycterium* shows a curious mixture of characters, some in advance of those of *Cheiropleuria*, others, and especially the distinctness of the sori, being more primitive.

The sum of the features noted in the above paragraphs appears to place *Platycterium* definitely in phyletic relation to the *Matonia-Dipteris* Series, as a form probably derived from such types, but curiously specialized; and perhaps its high degree of specialization to an epiphytic habit may account for the strange mixture of its characters. The anatomy of its stem relates it rather to *Matonia* and *Dipteris*; but its leaf, and its soral state points to *Cheiropleuria*. It may probably be held not to have been actually derived from any one of the living genera, but rather as a curiously specialized form, sprung from some extinct Matonioid or Dipterid which had a relatively advanced vascular structure; and that the soral system became specially enlarged, thereby giving a high spore-output, advantageous as an offset to the difficulties of an epiphytic habit.

The question remains whether any other Matonioid-Dipterid derivatives may be recognized among living genera. This question cannot be answered satisfactorily without much further comparative study. But it seems probable that others may actually be related. For instance, *Leptochilus tricuspidis* was placed close to *Cheiropleuria* by Sir W. Hooker. *Neocheiropteris* also is probably related, as well as some others ranked at present as species of '*Polypodium*'. A careful comparative study of such types, upon which I have already entered, would go far to decide what

can be at present no more than a suggestion of a possible relation of other living Ferns to the group under consideration.

It would thus appear that we may have in some measure to revise the current view of the Matonioid-Dipterid phylum. They are commonly held to have been a series of Gleichenioid origin, which was prevalent in the Mesozoic Period, but struggled on to the present time only in the surviving genera *Matonia* and *Dipteris*. There can now be little doubt that *Cheiropleuria* must be added to these surviving types, while *Platynerium* can hardly be anything else than a highly specialized Dipterid, related especially to *Cheiropleuria*, and adapted to modern life under the peculiar conditions of epiphytism. And it is possible that other derivative forms may be ultimately added to those modern representatives of a very ancient sequence.

Finally, if the position ascribed be accepted, the case of *Cheiropleuria* will have its value in showing that, in special cases, a want of parallelism of the several criteria is to be recognized and even expected. Many cases are already known. For instance, the single initial cells in stem and root of the Eu-sporangiate Ophioglossaceae, and the massive dermal appendages on the leaf-base of the primitive Gleichenias; the reticulate venation of the Eu-sporangiate *Kaulfussia*; the high subdivision of the vascular tracts in the living Marattiaceae; the protostelic structure combined with advanced gradate sori of the Hymenophyllaceae. But such instances of the absence of parallelism in the several criteria are relatively slight compared with the glaring fact of the primitive protostely, and simple hairs accompanying in *Cheiropleuria* a mixed sorus of an advanced 'Acrostichoid' type. It is this strange collocation of characters, which comparison marks out as incompatible elsewhere, that gives to *Cheiropleuria* its special interest and value in relation to the phyletic study of the Filicales.

#### SUMMARY.

1. *Cheiropleuria bicuspidis* (Bl.), Presl, is the only known species of a substantive genus.
2. It shows an uncommon mixture of primitive and advanced characters, by which it takes a place phyletically as a synthetic form.
3. Its characters, external and internal, connect it downwards most clearly with *Dipteris*; and upwards, that is in the direction of more advanced specialization, with *Platynerium*.
4. Its simple hairy investment, protostelic axis, undivided leaf-trace, and its frequently bifurcate form of leaf, are relatively primitive characters.
5. Its reticulate venation and its 'Acrostichoid' and 'mixed' sorus are characters of relative advance.
6. The occasional extension of the receptacular vascular supply of the individual sorus beyond the single vascular areola gives the clue to the

state of the sporophyll in *Platyserium*, with its double vascular system in the fertile region.

7. Its slightly oblique annulus, four-rowed sporangial stalk, and alternate segmentation of the primordium, form a peculiar link with *Dipteris*, which is shared also by *Metaxya*.

8. The mixed characters which this Fern shows are one of the clearest examples of non-parallelism of progression in the several criteria used for comparison among Ferns.

9. The outcome of its comparative examination is to strengthen the relation of *Dipteris* and *Matonia* to some Gleicheniacious source.

10. It shows that probably *Platyserium* is also a Dipterid derivative, specialized for an epiphytic habit.

11. Probably other Dipterid derivatives will also be found on detailed study, in such forms as *Leptochilus*, *Neocheiropteris*, and some others.

12. Thus the representation of Matonioid-Dipterid derivatives among living Ferns appears to be more extensive than had been hitherto appreciated.

## EXPLANATION OF FIGURES IN PLATES XXIV AND XXV.

Illustrating Professor Bower's Paper on *Cheiropleuria bicuspis* (Bl.) Presl, and allied genera.

### PLATE XXIV.

Fig. 1. Specimens of *Cheiropleuria bicuspis* (Bl.) Presl, from the Lingga Mountains, Borneo, showing the general habit, the one-cusped and two-cusped sterile leaves, and one narrow fertile leaf. Reduced to  $\frac{1}{4}$ .

Fig. 2 has been placed at the top of Plate XXV.

Fig. 3. Two sterile leaves of the bi-cusped type, as shown in Hooker's Figure. Journ. of Botany, 1846; also one fertile leaf. Reduced to  $\frac{1}{4}$ .

Figs. 4, 5. Specimens with irregularly furcate leaves. Reduced to  $\frac{1}{4}$ .

Fig. 6. A leaf of *Platyserium Hillii*, Moore, for comparison with Figs. 4 and 5. Reduced to  $\frac{1}{2}$ .

Fig. 7. Instead of this there has been substituted Text-fig. 1, which see.

Fig. 8. Transverse section of the protostele of *Gleichenia linearis*, Clarke (= *G. dichotoma*, Hk.), from a section by Professor Gwynne-Vaughan.  $\times 30$ .

Fig. 9. Transverse section of the protostele of *Gleichenia flabellata*, R. Br., from a section by Professor Gwynne-Vaughan, taken at a point where a leaf-trace is being given off. ( $\times 30$ ).

Fig. 10. Transverse section of a protostele of *Cheiropleuria*, showing a leaf-trace being given off from it.  $\times 45$ .

Fig. 11. A similar section of *Cheiropleuria*, showing the leaf-trace completely separated. It has two internal protoxylem-groups, with the metaxylem surrounding them completely—a condition which holds only for a short distance.  $\times 30$ .

Fig. 12. Transverse section of a young stele of *Cheiropleuria*, showing the absence of protoxylems, except in the young leaf-trace, while metaxylem tracheides are developing simultaneously, scattered through the whole area.  $\times 45$ .

Figs. 13, 14. Transverse sections showing the leaf-trace on its outward course, and beginning to bifurcate.  $\times 30$ .

Fig. 15. Transverse section of the stem of a seedling of *Dipteris conjugata*, Reinw, showing a protostelic state.  $\times 45$ .



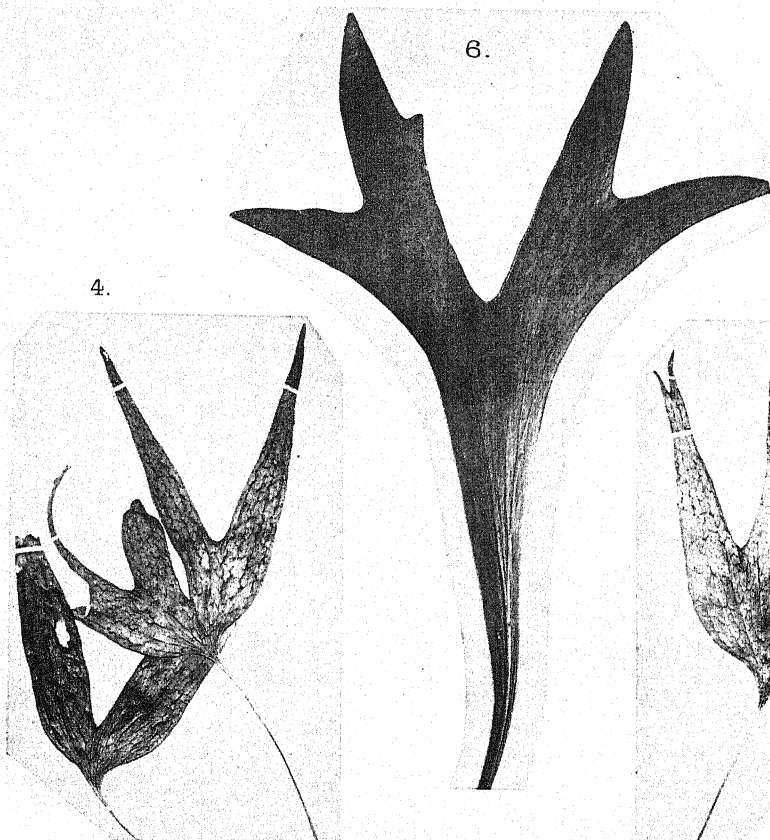
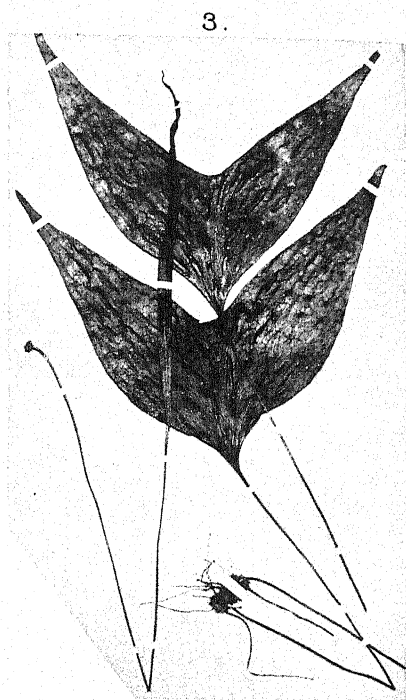
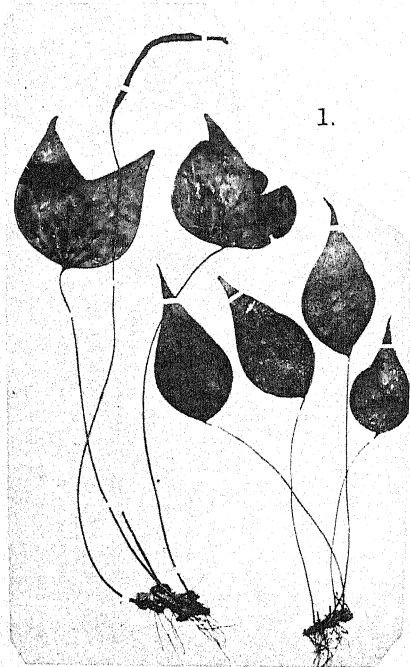
## PLATE XXV.

Fig. 2. Drawing by Mr. J. M. Thompson of a rhizome with the superficial hairs removed, so as to expose the successive leaf-bases, which are numbered *L*-i to *L*-viii, and the lateral axes which spring from their bases (*ax. i*, to *ax. iv*). But the leaves iii, vi, vii, viii have no associated axes. The leaf-arrangement is alternate, and the shoot is seen from the side facing away from the support.  $\times 2$ .

Fig. 16. Transverse section of a fertile leaf of *Platynerium Willinkii*, Moore, showing strands belonging to the two vascular systems: the strand nearer to the upper surface belongs to the main system of the lamina, that nearer the lower surface is a receptacular strand.  $\times 100$ .

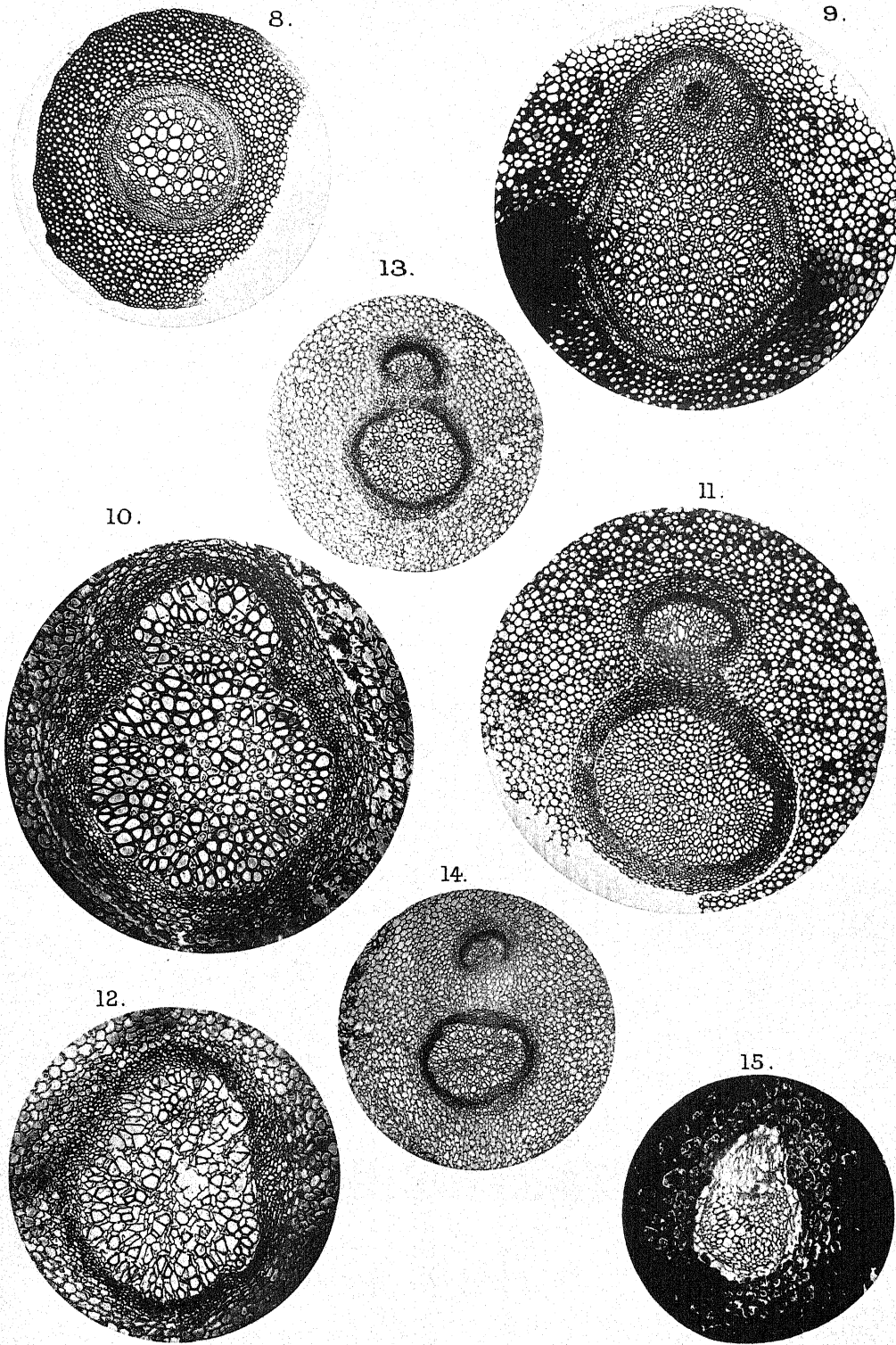
Fig. 17, *a-e*. Stages in the development of the sporangium of *Cheiropleuria*. *a*, *b*, *c* show the alternate cleavages of the primordium to form two rows of cells of the stalk: *d* shows the formation of the cap-cell: *e* shows the beginning of formation of the tapetum: *f* shows the tapetum complete, as two layers surrounding the sporogenous cell.  $\times 250$ .

Fig. 18, *a-c*. Stages in development of the sporangium of *Dipteris*. *a* shows a young sorus of *D. Lobbiana*, bearing two hairs, and two sporangia. The receptacular tracheides are closely below the surface, and are of the large storage type. *b* shows a sporangium of *D. conjugata* seen from the side facing one of the rows of segments, and demonstrating how each segment divides into two cells, which together with the results of division of the segments of the other row form the four rows of cells of the sporangial stalk. *c* represents transverse sections of similar sporangia of *D. conjugata*, showing some stalks—the younger, with only two cells; others, the older, with four cells. In one sporangium there are only three, one segment having divided and the other not.  $\times 325$ .



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BOWER-CHEIROPLEURIA.

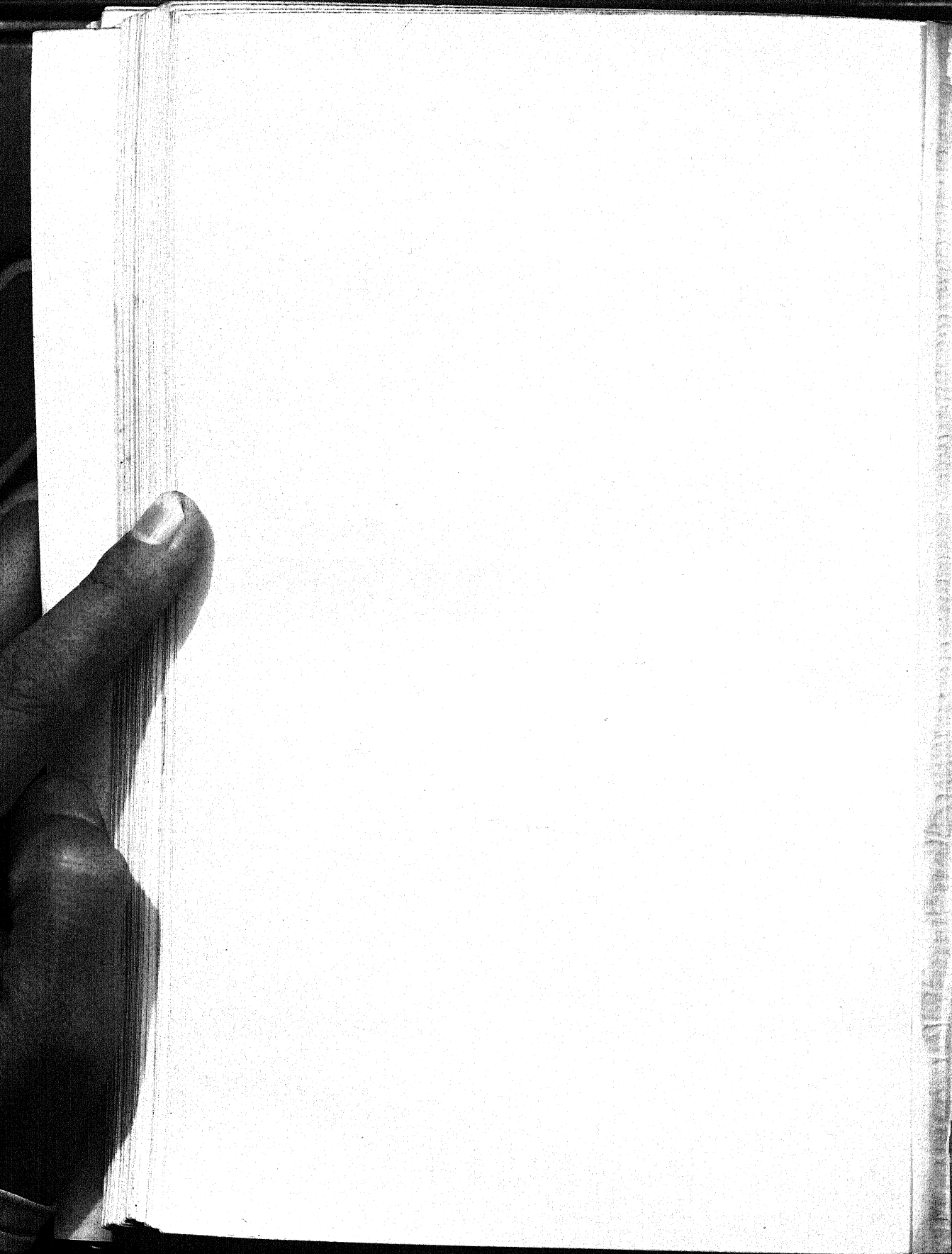


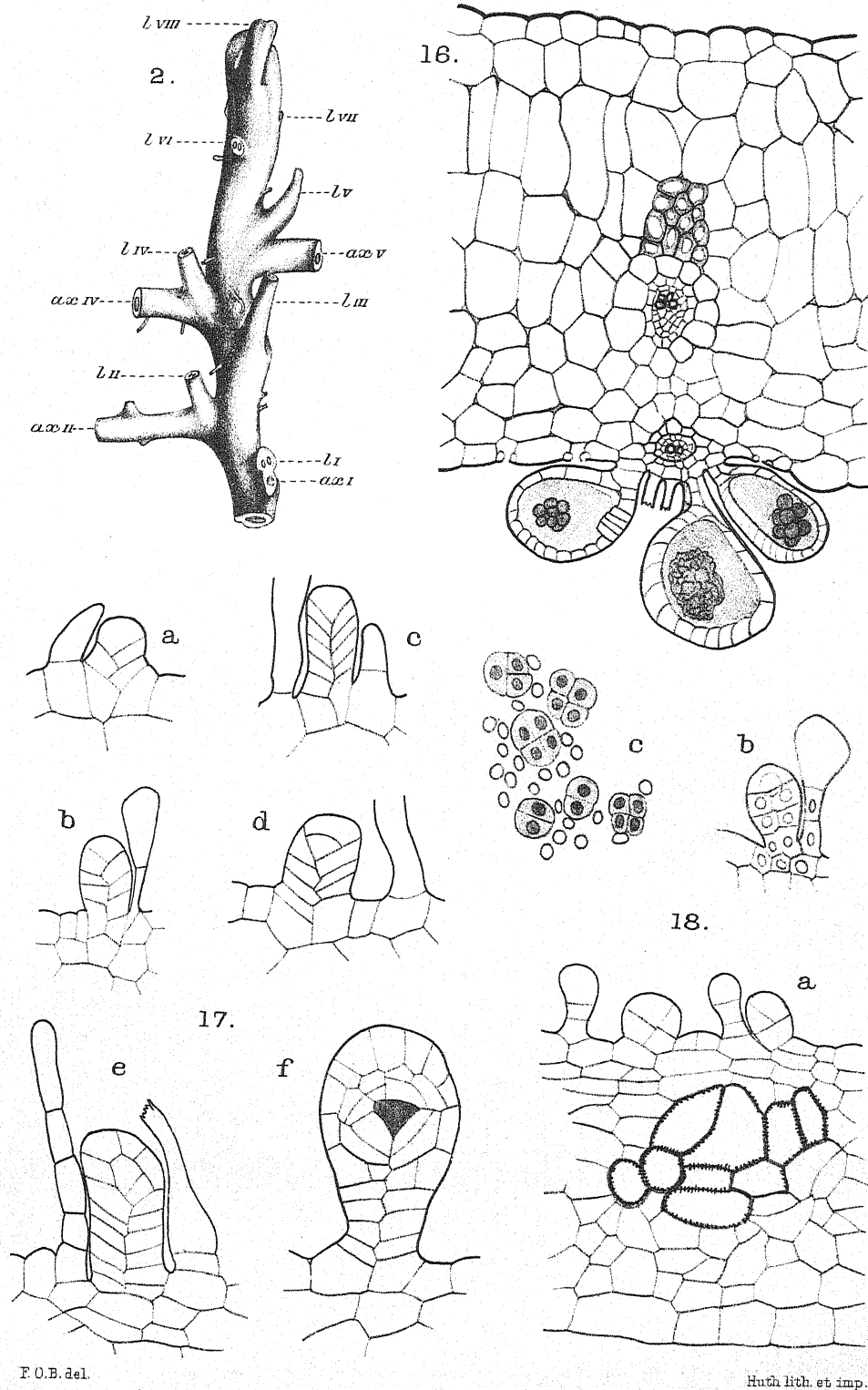
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# A Contribution to our Knowledge of *Rachiopteris cylindrica*, Will.

BY

N. BANCROFT.

With Plates XXVI and XXVII and seventeen Figures in the Text

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## I. SOURCES OF MATERIAL, AND ACKNOWLEDGEMENTS.

THE series of preparations of *Rachiopteris cylindrica* upon which the following description is mainly based, were handed to the writer by Prof. F. W. Oliver, to whom grateful acknowledgements are due, not only for the material, but for the opportunity to carry out this investigation at University College, London.

The writer also desires to thank Prof. F. E. Weiss, through whose kindness some valuable evidence has been obtained from the slides of the Cash and other collections, at Manchester University; Dr. A. Smith Woodward, for permission to examine and make drawings from the Williamson and General collections, at the British Museum; Prof. A. C. Seward and Dr. E. A. N. Arber, for similar opportunities with regard to



the Botany School and Binney collections, at Cambridge; Dr. W. T. Gordon, for the loan of slides, and for helpful discussion; Miss T. L. Pranker, for very kindly lending preparations and material of *Hottonia palustris* and *H. inflata*; and other friends who have supplied literature, and material of recent plants for comparative purposes.

## II. DISTRIBUTION AND HORIZON OF *R. cylindrica*.

The distribution of *Rachiopteris cylindrica* appears to be restricted to the Halifax-Huddersfield area, where it occurs in the Halifax Hard Bed of Lower Coal Measure Age.<sup>1</sup>

Williamson, who originally described the species in 1878,<sup>2</sup> remarked that he had never discovered it in the Oldham nodules; and a search through various collections comprising both old and recently acquired material has failed to reveal a single authentic specimen recorded from any other locality than that mentioned above.

In the case of a few specimens in the Williamson and Manchester collections no locality is given, but the general aspect and colour of the plant tissues indicate that this material also was obtained from the Halifax Hard Bed.

*R. cylindrica* is found in the nodules of the coal seam, and its excellent preservation suggests that it was petrified more or less *in situ*. The outer layers of the stem, it is true, are frequently somewhat crushed and eroded, but not more than is to be expected from compression and contact with other plant remains during turf formation.<sup>3</sup> In other cases, the outer tissues of the stem, with delicate hairs, are well preserved.

Doubtless, further working of the coal strata will extend our knowledge of the occurrence of *R. cylindrica*, but the material at present available indicates that this plant had a localized distribution.

## III. DETAILED DESCRIPTION OF THE ORGANS OF *R. cylindrica*.

An idea as to the structure and morphology of *Rachiopteris cylindrica* is drawn from the certain evidence of direct connexion between stems, roots, and primary petioles; and from the suggestive evidence of more or less constantly associated axial structures, and sporangia of Fern type. The first class of evidence, owing to the favourable preservation of the material, may be considered in detail.

<sup>1</sup> See Geological Ordnance Survey Map, No. 88 N.E.

<sup>2</sup> Williamson, W. C.: On the Organization of the Fossil Plants of the Coal Measures. Pt. ix, Phil. Trans. Roy. Soc., B, vol. 169, 1878, p. 319. See p. 351.

<sup>3</sup> Stopes, M. C., and Watson, D. M. S.: On the Present Distribution and Origin of the Calcareous Concretions in Coal Seams, known as 'Coal Balls'. Phil. Trans. Roy. Soc., B, vol. 200, 1908, p. 167. See p. 173



1. Stems.<sup>1</sup>

i. *General description*:—An examination of the serial sections of *R. cylindrica* gives the impression of a slender plant, the stems of which may have been prostrate upon the ground, or semi-erect, supporting themselves upon the surrounding vegetation.<sup>2</sup> These stems branched dichotomously<sup>3</sup> (Pl. XXVI, Figs. 5, 8, 9; Text-figs. 1, 7, 8) or produced leaves<sup>4</sup> (Pl. XXVI, Figs. 6 and 9; Text-figs. 8 and 9) at variable but fairly infrequent intervals, so that the habit of the plant must have been somewhat lax.<sup>5</sup> (See Text-figs. 1 and 8, in which the length of stem represented is indicated.) The relation of leaf production to dichotomy is variable; usually there is an interval between the two processes, although a leaf may occur in close association with a branch, as shown in Text-fig. 8.

Slender roots occur—usually singly—at or near the points of branching or leaf production (Pl. XXVI, Figs. 8 and 3).

The stems of *R. cylindrica* are circular in transverse section (Pl. XXVI, Figs. 2, 3, and 4), their average diameter being from 2 to 2.5 mm. The single central stele possesses a cylindrical core of wood (Pl. XXVI, Figs. 1-4), surrounded by phloem, in which the sieve-tubes are often distinct (Pl. XXVI, Fig. 2; Pl. XXVII, Fig. 4; Text-fig. 5); a somewhat irregular layer of cells, usually darkened, separates the stelar tissues from those of the cortex, and may be considered as an endodermis (Pl. XXVI, Fig. 1; Pl. XXVII, Fig. 4). The wood may have a single protoxylem group<sup>6</sup> (Pl. XXVI, Figs. 2, 3, and 7), in which case the structure is centrarch, or typically endarch; or there are from two to five groups (Pl. XXVI, Fig. 1; Pl. XXVII, Fig. 4; Text-fig. 2), when the structure tends towards mesarchy,

<sup>1</sup> For previous references see—

Williamson ('78), pp. 350, 351; Pl. 24, Figs. 80-88.

Hick, T.: On *Rachiopteris cylindrica*, Will. Mem. and Proc. Manchester Lit. and Phil. Soc., vol. 41, 1896, p. 1, Pl. I.

Tansley, A. G.: Lectures on the Evolution of the Filicinean Vascular System. New Phyt., Reprint, 1908. See p. 14 and Fig. 4.

Browne, Isabel: The Phylogeny and Inter-relationships of the Pteridophyta. New Phyt., Reprint, 1908. See p. 57.

Scott, D. H.: Studies in Fossil Botany. Second Edition, 1908. See p. 333.

Seward, A. C.: Fossil Plants, vol. 2, 1910. See pp. 438-40 and Fig. 305.

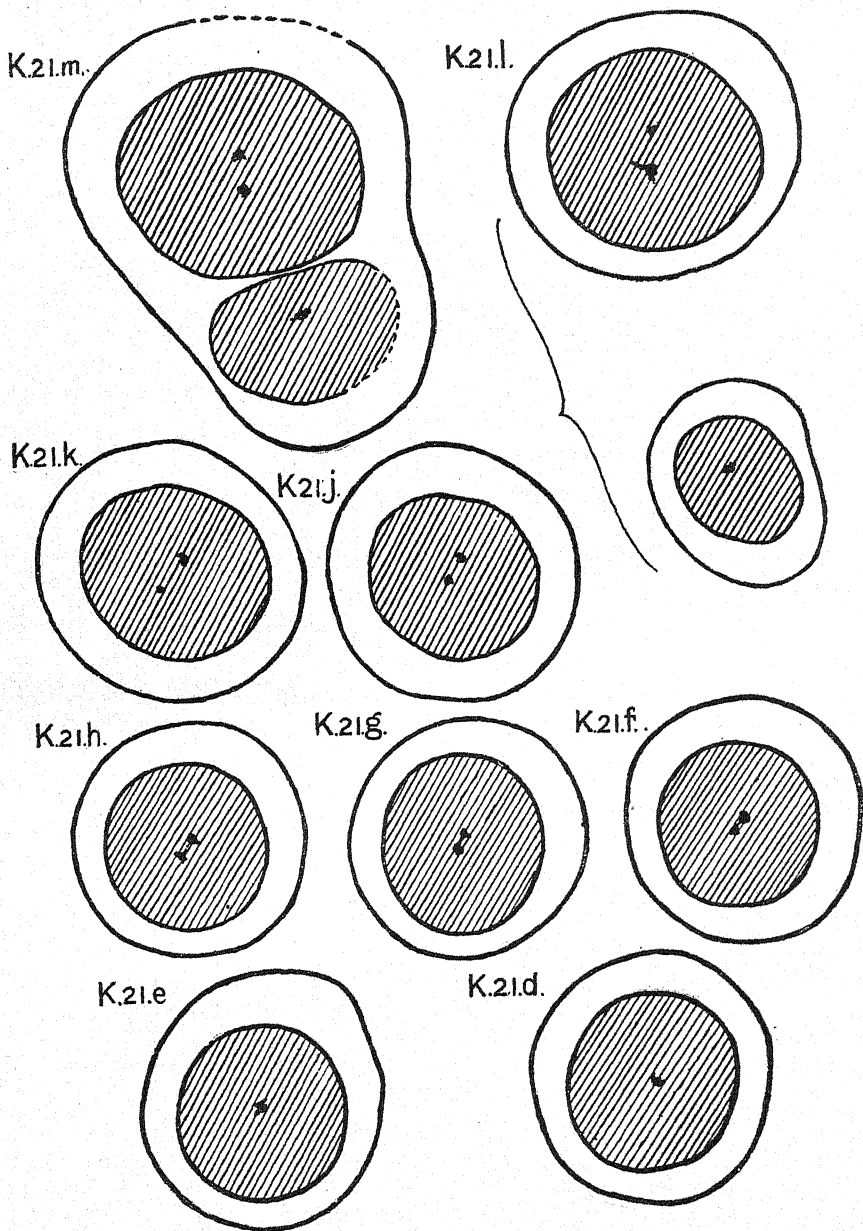
<sup>2</sup> See p. 554.

<sup>3</sup> This term is used provisionally and in a purely descriptive sense; as Dr. Lang has pointed out to the writer, it may be necessary to modify our views with regard to apparent dichotomy in these forms.

<sup>4</sup> Throughout the following account, the acropetal method of description is adopted, as being, in this case, the most convenient; see Boodle, L. A.: On Descriptions of Vascular Structures. New Phyt., vol. 2, 1903, p. 107. This author justifies the use of the acropetal method in descriptions of 'ferns, which have a solid cauline stele, or in which there are no leaf-traces showing distinct individuality in the internode', for 'in this way it is clearly seen what part of the stele is continuous with the leaf-trace' (p. 108).

<sup>5</sup> Tansley ('08), p. 15; Scott ('08), p. 333.

<sup>6</sup> That is, a group of small elements suggesting protoxylem; they do not seem to possess the typical annular or spiral thickenings.



TEXT-FIG. 1. Diagrams of the stele; a series of sections taken through about 1 inch of an  $\alpha$  stem, showing the occurrence of the centrarch condition above a place of branching. K 21 k-K 21 d represent the larger of the two steles in K 21 m and K 21 l, and show that the two original protoxylem groups fuse to form a single endarch strand. In this and other diagrams the xylem is shaded, and the protoxylem strands are indicated by black dots; a broken line represents a restored outline.  $\times 35$ . (From series K 21, stem B, University College, London.)

the groups being arranged round the centre, sometimes at considerable distances from it.<sup>1</sup> The stele is surrounded by a cortex, usually showing inner, middle, and outer regions (Pl. XXVI, Figs. 1 and 2); the epidermal layer, when not crushed, is seen to bear numerous unicellular, or typically multicellular hairs, often with somewhat rounded terminal cells (Text-fig. 4).

This general description applies to *all* stems referred to *R. cylindrica*; the stems, however, fall into two distinct groups, representative types of which will be described respectively as 'a' and 'β'. A discussion as to the significance of these two types will follow in Section V.<sup>2</sup>

ii. *a type*.<sup>3</sup>—The stems belonging to this group are characterized by the possession of large xylem strands, the average diameter of which is 0.7 mm. There are typically several (two, or, more commonly, from three to five) protoxylem groups arranged round the centre (Pl. XXVI, Fig. 1); the centrarch condition with one protoxylem group occurs (Pl. XXVI, Fig. 2), but it is comparatively rare, and results from the fusion of two or more groups (Text-fig. 1). The normal condition in stems of *a* type thus tends towards mesarchy, for the occurrence of more than two protoxylem groups is quite independent of branching,<sup>4</sup> as an examination of serial sections demonstrates (Text-fig. 7).

The great interest of this type lies in the fact that the mesarch condition is accompanied in many cases by a certain amount of differentiation of the xylem<sup>5</sup> into an outer zone in which the lumen of the tracheides is of ordinary size (100 μ in diameter), and an inner solid core, in which the tracheides are much narrower (Text-fig. 2; Pl. XXVI, Fig. 1). There are no parenchymatous cells interspersed among the tracheides.<sup>6</sup> The differentiation of the wood is particularly marked in the larger stems (Text-fig. 2), transverse sections of which recall the condition seen in *Diplolabis Römeri*.<sup>7</sup> The protoxylem groups are situated at the junction of the wide and narrow elements (Text-fig. 2), as in *Diplolabis*. The size of the core of small elements is very variable (cf. Pl. XXVI, Fig. 1, and Text-fig. 2); it is dependent on the size of the xylem strand as a whole, on the number of protoxylem groups present, and on their distance from the centre of the strand.

<sup>1</sup> With regard to this point, cf. the statements of Williamson ('78), p. 350; Hick ('96), p. 8; Tansley ('08), p. 15; Scott ('08), p. 333; Brown ('08), p. 57; Seward ('10), p. 438. The general view is that *R. cylindrica* is typically endarch; Hick noted the frequent presence of four or five protoxylem groups arranged symmetrically round the centre of the stele, but Lady Isabel Browne was the first author to comment upon this tendency towards mesarchy.

<sup>2</sup> p. 553.

<sup>3</sup> Williamson based his original description upon this type: ('78), p. 350, Pl. 24, Figs. 80 and 87.

<sup>4</sup> Cf. Browne ('08), p. 57.

<sup>5</sup> Gordon, W. T.: On the Relation between the Fossil Osmundaceae and the Zygopterideae. Proc. Cambridge Phil. Soc., vol. 15, Pt. V, 1910, p. 398. See p. 400.

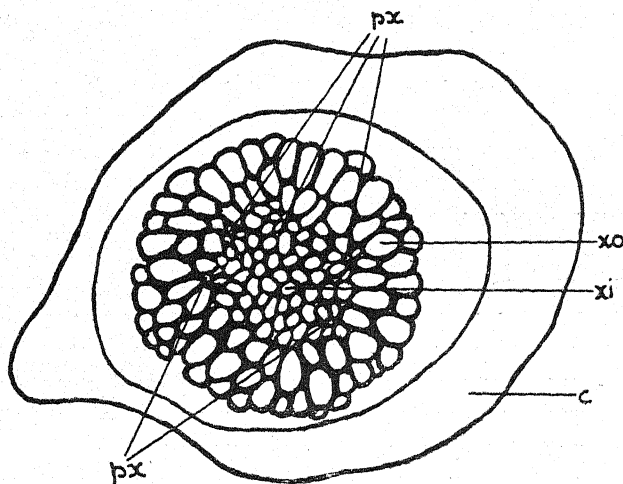
<sup>6</sup> Hick ('96), p. 9.

<sup>7</sup> Gordon, W. T.: On the Structure and Affinities of *Diplolabis Römeri* (Solms). Trans. Roy. Soc. Edin., vol. 47, Pt. IV, 1911, p. 711. See p. 719.

Stems of *a* type are further characterized by the structure of the cortex, which presents three areas, though these are not always distinctly recognizable, owing to the tendency of the outer cortex to become crushed and eroded (Pl. XXVI, Figs. 1 and 2).

The cells composing from three to six of the innermost cortical layers are somewhat rectangular and tangentially elongated as seen in transverse section. They have thick walls, and the layers are often arranged more or less concentrically<sup>1</sup> in a manner more characteristic of roots than of stems (Pl. XXVI, Figs. 1 and 2).

The inner cortex passes gradually into the middle cortex, in which the



TEXT-FIG. 2. A large *a* stem, in which the xylem is shown in detail. Note the distinct differentiation of the xylem into large outer (*xo.*), and small inner (*xi.*) tracheides. There are five groups of tracheides (*px.*) suggesting protoxylem, situated at the junction of the outer and inner wood. The cortical layers (*c.*) of this stem are much crushed.  $\times 40$ . (From slide 1552, Williamson collection.)

cells are rather loosely arranged and are more rounded in transverse section than those of the preceding area (Pl. XXVI, Figs. 1 and 2). They are irregular in size, and the thickness of their walls is slightly variable in different specimens.

The outer cortex is composed of from four to twelve layers of fairly large thin-walled cells (Text-fig. 3), and is thus markedly distinct from the middle cortex, forming a delicate tissue which is usually more or less crushed, so that the form of the component cells is unrecognizable (Pl. XXVI, Figs. 1 and 2). The appearance of this zone suggests that it may have formed an assimilatory tissue comparable with that of the stems of *Psilotum*, or the rachis of *Stauropteris*.<sup>2</sup> Occasionally the whole of the

<sup>1</sup> Williamson ('78), p. 350; Hick ('06), p. 3.

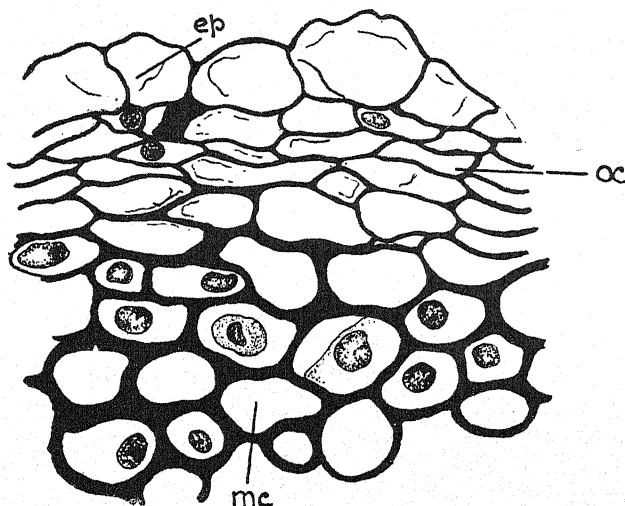
<sup>2</sup> Scott, D. H.: The Sporangia of *Stauropteris oldhamia*. New Phyt., vol. 4, 1905, p. 114. See p. 115.

Bertrand, P.: Études sur la Fronde des Zygoptéridées. Lille, 1909. See Pl. VII, Fig. 48.

outer cortex appears to be obliterated, and in these cases, the cortex as a whole presents a fairly uniform appearance, the rounded cells of the middle cortex being the most conspicuous feature.

In longitudinal section the inner and middle cortical cells are elongated, their end walls being straight or oblique; no specimen has been observed which gives a clear idea of the outer cortical cells in longitudinal section.

In some specimens large cavities occur here and there in the outer cortex and particularly where this adjoins the middle cortex (Pl. XXVI, Figs. 1 and 5). Their general appearance suggests that they may have been secretory in function, though no trace of contents has been observed. On



TEXT-FIG. 3. The outer layers of an  $\alpha$  stem, just above a branch, showing the thin-walled cells of the outer cortex (*oc.*), and the irregular epidermal cells (*ep.*) still uncrushed; *mc.*, middle cortical cells.  $\times 400$ . (From slide K 20 m, University College, London.)

the other hand, they may merely be due to breaking down of the cells before or during petrification.

The epidermal cells are rarely recognizable<sup>1</sup> in stems of  $\alpha$  type. In some cases, however, at a place of branching, the structure of the epidermis is well shown on the inner or 'separation' surface of both products of stem division (Text-fig. 4, *a* and *b*). The epidermal cells are large and rather irregular in shape, as seen in transverse section, while in longitudinal section they appear to be slightly elongated; frequently they produce multicellular hairs (Text-fig. 4, *b* and *c*).

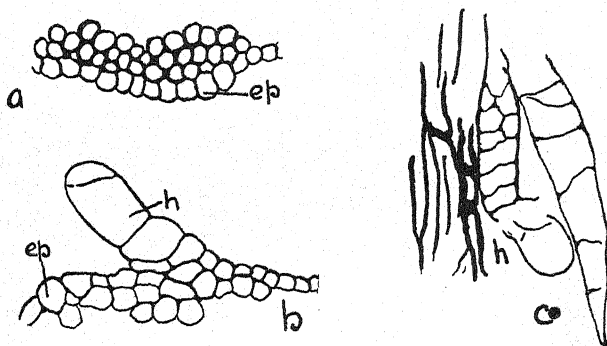
A point of interest is the absence of the thin-walled cortical cells under the epidermis, in the region just above the separation of a branch (Text-fig. 4, *a* and *b*). These seem to develop gradually, becoming intercalated between the thicker-walled cells and the epidermis. Text-fig. 3 shows

<sup>1</sup> Hick ('96), p. 2.

a stage in the production of the outer cortex just above branch separation. The large-celled epidermis is still distinct at this point ; no traces of stomata have been observed.

iii.  $\beta$  type.—The stems of *R. cylindrica* described and figured by Hick are, with one exception, referable to this type<sup>1</sup> ; the occurrence of the two forms is not specially mentioned by this author.<sup>2</sup>

Stems of  $\beta$  type are similar in size to those of  $\alpha$  type, but are distinguished from them by the relatively small size of the cylindrical xylem strand, of which the average diameter is 0.4 mm. This strand typically possesses a single central protoxylem group, so that a condition of true endarchy is realized (Pl. XXVI, Figs. 3 and 7). The occurrence of more than one protoxylem in this case seems normally to be in connexion with branch-



TEXT-FIG. 4. *a*, Separation surface of an  $\alpha$  petiole, showing the epidermis (*ep*). Note the absence of the narrow, thin-walled cells underlying the epidermis (cf. Fig. 3). *b*, separation surface of an  $\alpha$  stem, showing the irregular epidermis, and an epidermal hair (*h*). The narrow, thin-walled cells are absent here also. *c*, a portion of an oblique longitudinal section of an  $\alpha$  stem, showing two hairs of which the connexion with the epidermis cannot be observed.  $\times 80$ . (*a*, and *b*, from slide K 20 *m* ; *c*, from slide K 21 *h*, University College, London.)

ing (Text-fig. 8) ; and apart from the narrow elements of the protoxylem group, there is no differentiation in size between central and outer tracheides. The elements are all of the type of the outer tracheides in  $\alpha$  stems, although they are less in diameter ( $70\mu$ ) (cf. Pl. XXVI, Figs. 1 and 8).

The three cortical areas are again distinguishable in  $\beta$  type stems.<sup>3</sup>

The inner cortex is composed of two or three series of tangentially elongated cells (Pl. XXVI, Figs. 3, 8, and 9), the walls of which are firm but not thickened to any extent. There is a varying tendency towards concentric arrangement of the inner cell-series, and the gradual transition to the middle cortex takes place as in  $\alpha$  stems.

The middle cortex consists of rounded or polygonal cells, thin-walled, and somewhat irregular in size. Near the inner limit of this area is a band

<sup>1</sup> Hick ('96) : the exception is figured in Pl. I, Fig. 5.

<sup>2</sup> Williamson figures and notes the two forms of stem ; his Fig. 88 is a  $\beta$  type stem.

<sup>3</sup> Cf. Hick's description ('96), p. 4.

consisting of several cell-layers in which the elements are small and very loosely arranged (Pl. XXVI, Figs. 3 and 8). In some specimens this band of cells has become crushed (Pl. XXVI, Fig. 9), in others it has given rise to radial lacunae (Pl. XXVI, Fig. 4), the presence of which was noted and figured by Hick.<sup>1</sup> Beyond the lacunae, the cells of the middle cortex are larger, and tend to become somewhat radially elongated (Pl. XXVI, Figs. 3 and 7); the outermost cells of this area have slightly thickened walls (Pl. XXVI, Figs. 3, 4, and 7).

The outer cortical cells are apparently of the same type as those of  $\alpha$  stems (Pl. XXVI, Fig. 8), although they are usually much more crushed. Occasionally the epidermis, with numerous hairs, may be observed, but in these cases the thin-walled cortex is not developed.

In longitudinal section the thicker-walled cortical cells are elongated; the thin-walled elements of the middle cortex are shorter, having their end walls more or less horizontal.

iv. *Histology*.—The unusually favourable preservation of *R. cylindrica* permits a fuller investigation of its histology than is possible in many cases.

The details of cell structure in  $\alpha$  and  $\beta$  stems are similar. The long, pointed tracheides are pitted on all surfaces; and while the narrower walls present a typically scalariform appearance, the broader walls are reticulately pitted. The arrangement and extent of the pits may occasionally be observed in transverse section.<sup>2</sup>

The smaller tracheides, considered as representing protoxylem elements, are usually pitted in a scalariform manner,<sup>3</sup> instead of showing the typical annular or spiral thickening; this fact suggests that the growth of the stems was not rapid.<sup>4</sup>

There are a few scattered cells of xylem parenchyma, often with their contents preserved, and a continuous zone of phloem surrounds the xylem strand (Text-fig. 5). The phloem typically consists of a layer of large sieve-tubes accompanied on either side by narrow cells of phloem parenchyma. The sieve-tubes usually form a single series, and are as distinct in transverse section (Text-fig. 5, *st.*; Pl. XXVII, Fig. 4) as the corresponding elements in a living Fern, such as *Pteridium* or *Marsilia*.<sup>5</sup> It has been impossible to obtain a clear idea of the phloem in longitudinal section, and a careful search has failed to reveal any trace of sieve-plates.

<sup>1</sup> I. c., Pl. I, Fig. 1 represents the stem in Slide Q 104, Cash collection; Pl. XXVI, Fig. 4 of the present account is a photograph of the stem in Slide Q 103, cut from the same block as Q 104.

<sup>2</sup> Pl. XXVII, Fig. 1 shows the pits in transverse section in the case of a petiole.

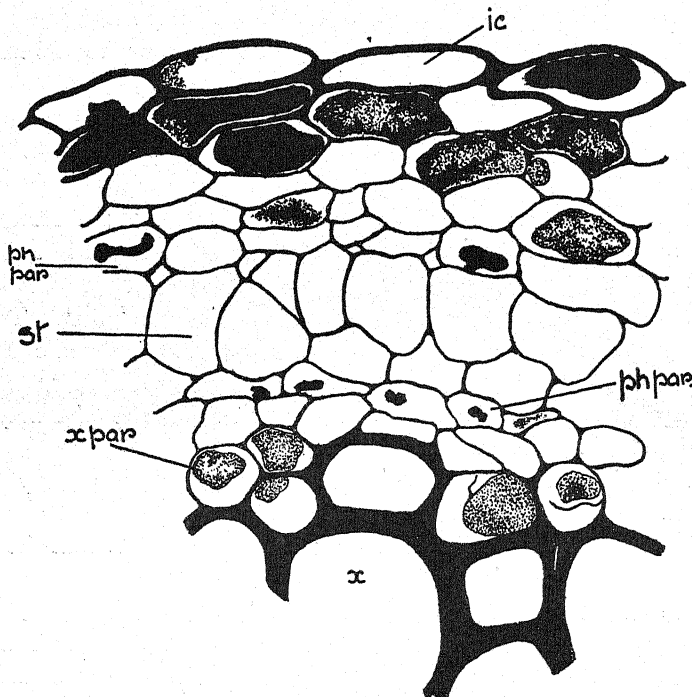
<sup>3</sup> Cf. Hick ('96), p. 8; the suggestion of spiral elements is very doubtful. Tansley ('08), pp. 14 and 15.

<sup>4</sup> Tansley ('08), p. 12. Scott ('08), p. 329.

<sup>5</sup> Hume, E. M. M.: The Histology of the Sieve-tubes of *Pteridium aquilinum*, with some notes on *Marsilia quadrifolia* and *Lygodium dichotomum*. Ann. of Bot., vol. 26, 1912, p. 573. See pp. 576 and 577, and Pl. LV, Figs. 31 and 32.

In some cases, sieve-tubes are not present, or the ring may be incomplete; in these cases the phloem consists of a uniform zone of smaller, narrower cells.<sup>1</sup> On the whole, the ring of sieve-tubes is less marked in  $\beta$  type than in  $\alpha$  type stems.

In some well-preserved examples a clearly-defined continuous layer of cells external to the phloem may be considered as representing the pericycle<sup>2</sup> (Pl. XXVII, Fig. 4); it is, however, frequently impossible to refer the outer tissues of the stele to a definite series (Text-fig. 5).



TEXT-FIG. 5. A portion of the stele of an  $\alpha$  stem, showing the xylem ( $x$ ), xylem parenchyma ( $x.par.$ ), sieve-tubes ( $st.$ ), phloem parenchyma ( $ph.par.$ ). The outermost layer of cells ( $ic.$ ) belongs to the inner cortex, having the typical radially-compressed form. Endodermis and pericycle do not constitute distinct layers in this specimen.  $\times 400$ . (From slide K 20  $h$ , University College, London.)

There is some doubt as to the existence of a true endodermis in stems of *R. cylindrica*.<sup>3</sup> The stele is usually limited by a somewhat irregular layer of dark-coloured cells, fairly thin-walled, and elongated both tangentially and vertically. This layer, particularly in  $\alpha$  stems, sometimes has

<sup>1</sup> Hick ('96), p. 7, notes the apparent lack of differentiation of the phloem cells, but mentions that elements resembling sieve-tubes occur in specimen Q 104.

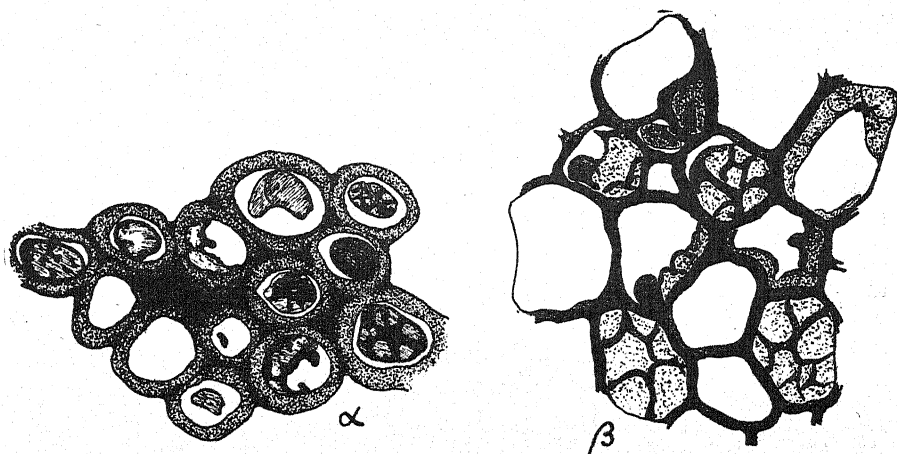
Tansley ('08), p. 14, Fig. 4, shows sieve-tubes in specimen K 14, University College collection. In Williamson's figure ('78, Pl. 24, Fig. 80) there are no sieve-tubes; see p. 350.

<sup>2</sup> Hick ('96), p. 7.

<sup>3</sup> *Ib.*, p. 6.



the appearance of a sharply defined bundle sheath <sup>1</sup> (Pl. XXVII, Fig. 4, *e*), the constituent elements of which alternate with those of the pericycle, and also with the cortical cells succeeding them. The distinctness of this darkened layer is very variable, and, particularly in the case of  $\beta$  stems, it is sometimes so ill-defined that the stelar tissues appear to grade into those of the cortex without any reliable indication of a limiting layer. The dark colour of these cells seems to be due to the blackening of the contents, which must have been very dense. Sometimes the cells appear uniformly dark, as if the colouring belonged entirely to the walls; in these cases there can have been little or no contraction of the cell contents. At other times the blackened contents have contracted away from the walls, locally or entirely; <sup>2</sup>



TEXT-FIG. 6. Portions of the middle cortex of  $\alpha$  and  $\beta$  stems, showing the difference in the thickness of the cell-walls, and the appearances assumed by the contracted cell-contents.  $\times 400$ . ( $\alpha$ , from Q 101;  $\beta$ , from Q 103, Cash collection.)

while in still other cases the cells possess a pitted and reticulate appearance, suggesting uneven adhesion of the contents to the cell-wall, so that subsequent contraction has caused ruptures which now appear as spaces or pits. This appearance may be observed in longitudinal as well as in transverse section; <sup>3</sup> whether it is natural, or due to a condition of petrifact, <sup>4</sup> cannot of course be determined.

<sup>1</sup> Williamson ('78), p. 350; Pl. 24, Fig. 80.

<sup>2</sup> Cf. Scott ('08), p. 329, Fig. 122. In this figure of *Botryopteris hirsuta*, contraction of the contents may be observed in the darkened cell-layer which 'may be the endodermis'.

Kidston, R.: On a New Species of *Dineuron* and of *Botryopteris* from Pettycur, Fife. Trans. Roy. Soc. Edin., vol. 46, Pt. II, 1909, p. 361. See p. 363: 'the endodermis (of *B. antiqua*) is clearly defined by its dark contents.'

<sup>3</sup> The endodermis of the stem is exactly similar to that of the root, figured in Pl. XXVII, Fig. 3 and Text-figs. 12 and 13.

<sup>4</sup> Stopes, M. C.: Petrifications of the earliest European Angiosperms. Phil. Trans. Roy. Soc., B, vol. 203, 1912, p. 75. See p. 94.

Frequently in  $\alpha$  stems and occasionally in those of  $\beta$  type the contents of the cortical cells are preserved (Pl. XXVI, Figs. 1 and 4, and Text-fig. 6), showing very varied conditions of blackening, granulation, and contraction; here again the appearances may be due to petrifact, although Hick<sup>1</sup> is inclined to attach some systematic importance to them. On the whole the cortical cell-contents of  $\beta$  stems are less blackened than those of  $\alpha$  stems; representative examples are shown in Text-fig. 6.

With regard to the epidermal cells and hairs, Hick<sup>2</sup> has remarked that no contents are recognizable. In cases, however, where the cells are preserved in a growing state, as they are just above a place of branching, delicate, granular and vesicular contents may sometimes be detected. The multicellular hairs, when fully grown, appear to be appressed rather than spreading, and consist usually of a single row of cells, the terminal member of which may be pointed or rounded (Text-fig. 4, *c*). The terminal cells of young hairs often present the appearance of glands, particularly when contents are recognizable. In some cases a hair appears to grow from a single basal cell; frequently, however, it is carried by a pedestal consisting of several small cells<sup>3</sup> (Text-fig. 4, *b*). In one of the examples figured the lower part of the hair itself consists of a double row of cells (Text-fig. 4, *c*), suggesting a transitional state between typical uniseriate hairs and flattened multiseriate rammenta.

*v. Branching.*—‘Dichotomy’ of the stems, described by Hick<sup>4</sup> as ‘equal division’, takes place in both  $\alpha$  and  $\beta$  types. In  $\beta$  stems the single protoxylem group divides, and in passing up the stem<sup>5</sup> the two resultant groups become more and more separated as the xylem strand increases in size (Text-fig. 8 and Pl. XXVI, Fig. 9). At the level where it presents about twice its original dimensions, narrow thin-walled cells occur laterally, and their gradual inward extension separates two equal, or nearly equal, masses of tracheides, each having typically a single central protoxylem group (Pl. XXVI, Fig. 8; Text-fig. 8).<sup>6</sup> As the strands separate in passing upwards, each becomes surrounded by phloem and endodermis, so that the stem, just below the actual bifurcation, contains two similar steles which are destined for the two branches of the dichotomy (Pl. XXVI, Fig. 8; Text-fig. 8, K 21 r).

In the division of typical  $\alpha$  stems possessing several protoxylem groups, only one of these is concerned in the production of the branch, the initial strand of which seems to be provided by the division of that group<sup>7</sup>

<sup>1</sup> l. c., pp. 4–6.

<sup>2</sup> l. c., pp. 2 and 3.

<sup>3</sup> Hick ('96), p. 2.

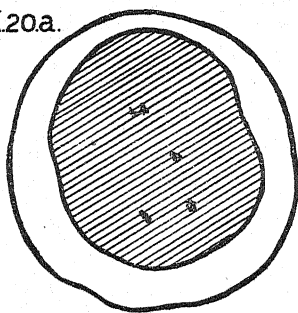
<sup>4</sup> l. c., p. 9; Pl. I, Fig. 2. Scott ('08), p. 333.

<sup>5</sup> See foot-note 4, p. 533, concerning the acropetal method of description. In the paper quoted, Boodle also justifies the use of words signifying motion of vascular structures, as ‘avoiding a lengthy periphrasis’ (page 107, foot-note 2).

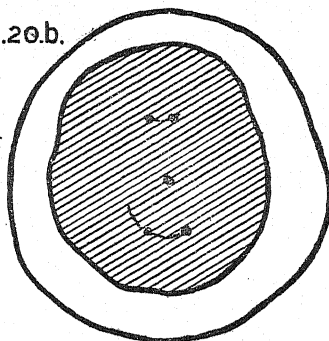
<sup>6</sup> In Text-fig. 553, the occurrence of two protoxylem groups in one of the branch-steles is not typical; it appears rather to be a transitory condition than to have any connexion with branching.

<sup>7</sup> In the stem figured, the position of the protoxylem groups indicates the division of one of them, lower in the stem, to supply the branch, although none of the series examined shows the actual process.

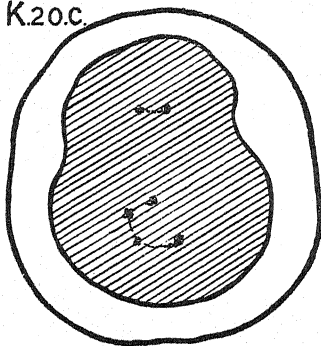
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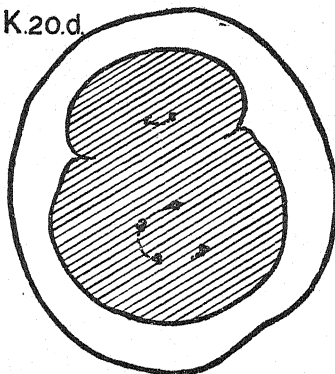
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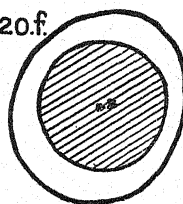
K.20c.



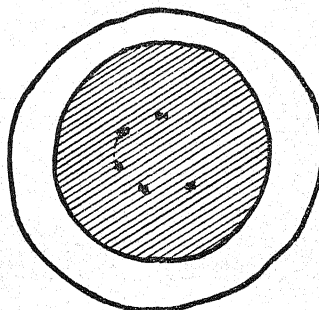
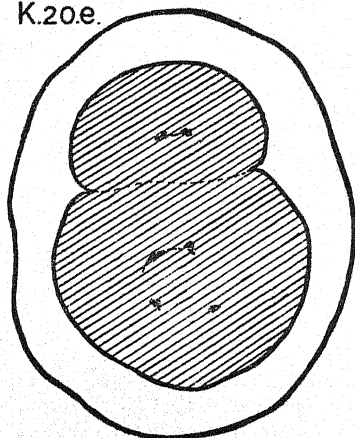
K.20d.



K.20f.

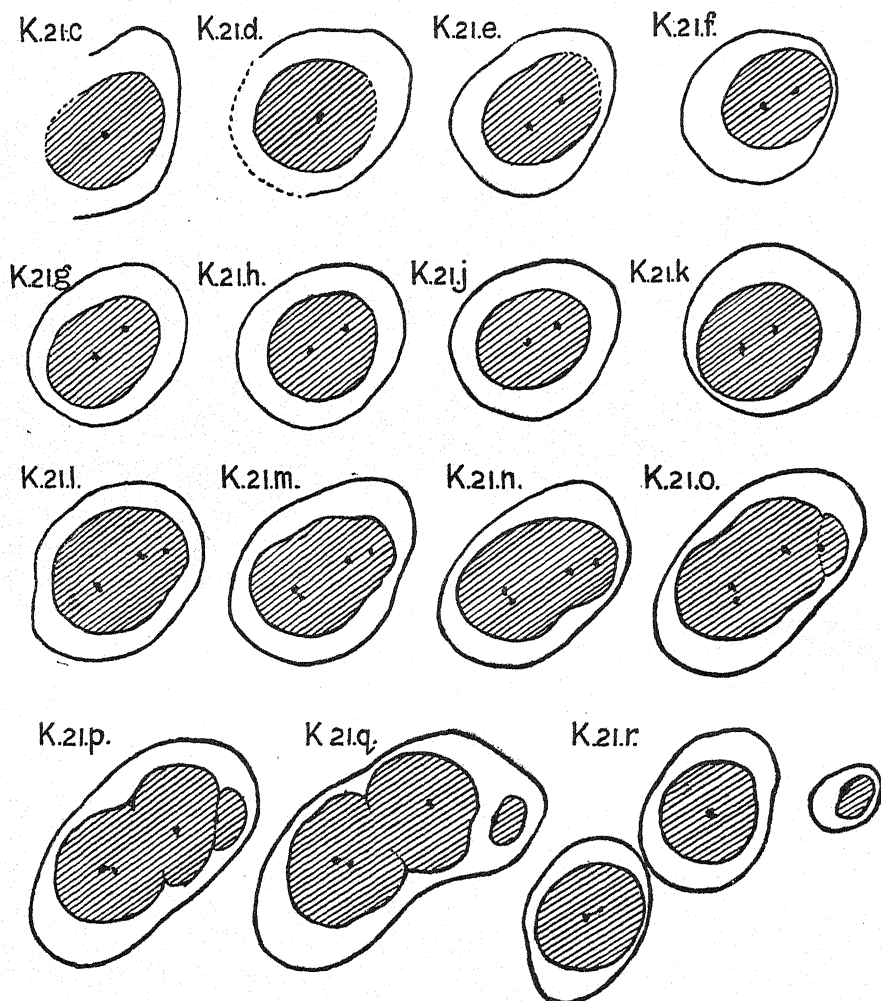


K.20e.



TEXT-FIG. 7. Diagrams of the stele of an  $\alpha$  stem, showing 'dichotomy'; the branch stele is considerably smaller than the parent stele. Note the mesarch position of the protoxylem in the large stele, and the occurrence of two strands in the branch even at a low level.  $\times 35$ . (From series K 20, University College, London.)

(Text-fig. 7). Formation and separation of the two steles are similar to the processes described for  $\beta$  stems; below the level of complete separation, the single protoxylem group of the branch may divide once or twice (Pl. XXVI, Fig. 5; Text-fig. 7).



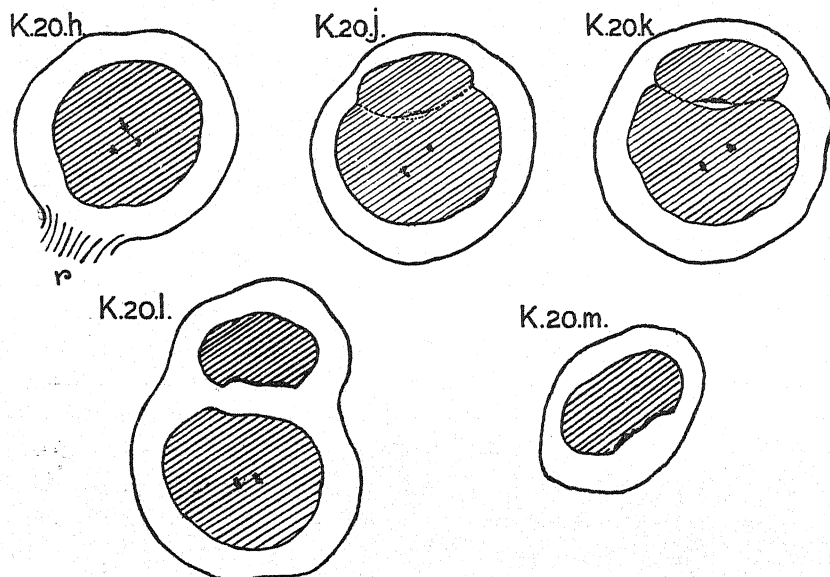
TEXT-FIG. 8. Diagrams of the stele; a series of sections taken through about  $1\frac{1}{2}$  inches of a  $\beta$  stem, showing a 'dichotomy' in which the two branches are equal, and also the emission of a monarch foliar-trace. Compare the size of these steles with those of  $\alpha$  stems, drawn to the same scale.  $\times 35$ . (From series K 21, stem C, University College, London.)

In branching  $\alpha$  stems, although the two products are similar in structure, one is usually smaller than the other (Pl. XXVI, Fig. 5; Text-figs. 1 and 7); indeed the division can hardly be termed 'equal', especially as only one of the several original protoxylem groups participates in branch

formation. In  $\beta$  stems the branches are typically equal in size (Pl. XXVI, Figs. 8 and 9; Text-fig. 8).

## 2. Petioles.

i. *Development*.—The production of petioles has been described by Hick as a process of unequal division of the stem.<sup>1</sup> The lowermost stages of their development are exactly similar to those seen in the formation of stem branches; the division of the single, or one of the several, protoxylem



TEXT-FIG. 9. Diagrams of leaf-trace production in the smaller branch of the  $\alpha$  stem shown in Text-fig. 7. In K 20*h* indications of root-production are seen at *r*; K 20*j* and K 20*k* indicate the appearance of separating cells immediately in front of the protoxylem of the leaf-trace; K 20*l* shows the position of the fully-formed petiolar bundle as compared with that of the trace in K 20*h*. Note the distribution of the protoxylem along the anterior surface of the leaf-trace, and the absence of adaxial metaxylem.  $\times 35$ . (From series K 20, University College, London.)

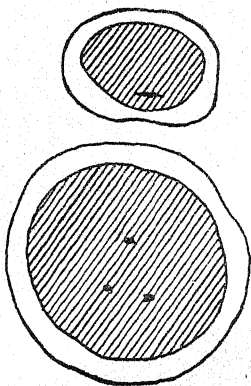
groups (Text-figs. 8 and 9) provides the initial petiolar strand, which in passing upwards becomes separated from the rest by the formation of metaxylem elements (Text-figs. 8 and 9). At a slightly higher level in the stem, thin-walled parenchymatous cells may be observed in front of the petiolar protoxylem (Text-fig. 9; Pl. XXVI, Fig. 6); higher still, the lateral extension of these cells completely separates the semi-cylindrical mass of foliar xylem from the stem stele. Always in  $\beta$  types, and in some  $\alpha$  types also, the protoxylem occurs on the adaxial margin of the petiolar strand (Text-figs. 8 and 9). In other  $\alpha$  types the protoxylem, just above the level of trace-separation, is slightly immersed, owing to the occurrence of some

<sup>1</sup> l. c., pp. 9-11.

adaxial metaxylem elements (Pl. XXVII, Fig. 1; Text-fig. 10).<sup>1</sup> The endodermis and the remaining stelar tissues close round the xylem, and the leaf-base separates from the stem in the same way as an ordinary branch.

In its outward passage the petiolar bundle may turn slightly away from the stem stele, this deflection being apparently more marked in  $\alpha$  than in  $\beta$  types (cf. Text-fig. 9 with Text-fig. 8, and Pl. XXVI, Figs. 3 and 7).

ii.  $\alpha$  petioles.—These are circular in outline and are smaller than the stems, having an average diameter of 1.5 mm. They each possess a single xylem strand—0.45 mm.  $\times$  0.3 mm. in average dimension—which varies in shape from semilunar, at its lower levels, to elliptical or bluntly oblong as



TEXT-FIG. 10. Diagram of a leaf-trace just above its separation from the stem-stele. Note the undivided protoxylem group of the leaf-trace, and the occurrence of adaxial metaxylem.  $\times 35$ . (From slide 187, Williamson collect.)

seen in transverse section; there is occasionally a slight indentation on the abaxial margin of the strand (Text-fig. 11,  $a$ ). The position and behaviour of the protoxylem elements is exceedingly variable. The initial group always exhibits a tendency to divide, and typically there is a definite division at some point above the separation of the petiolar strand from that of the stem; the resulting condition of diarchy, however (Text-fig. 11), does not appear to set in at any fixed level. As the leaf-base passes away from the stem, the adaxial metaxylem elements, when present, tend to break down and disappear (Pl. XXVII, Fig. 4; see also Text-fig. 11), so that the protoxylem is again external at a slightly higher level in the petiole. It may appear to occupy two shallow grooves, or to form two blunt points (Text-fig. 11,  $a$  and  $b$ ). In cases where there is no definite division of the protoxylem group, the more or less flattened

adaxial margin of the xylem seems to consist almost entirely of small elements<sup>2</sup> (Text-fig. 9).

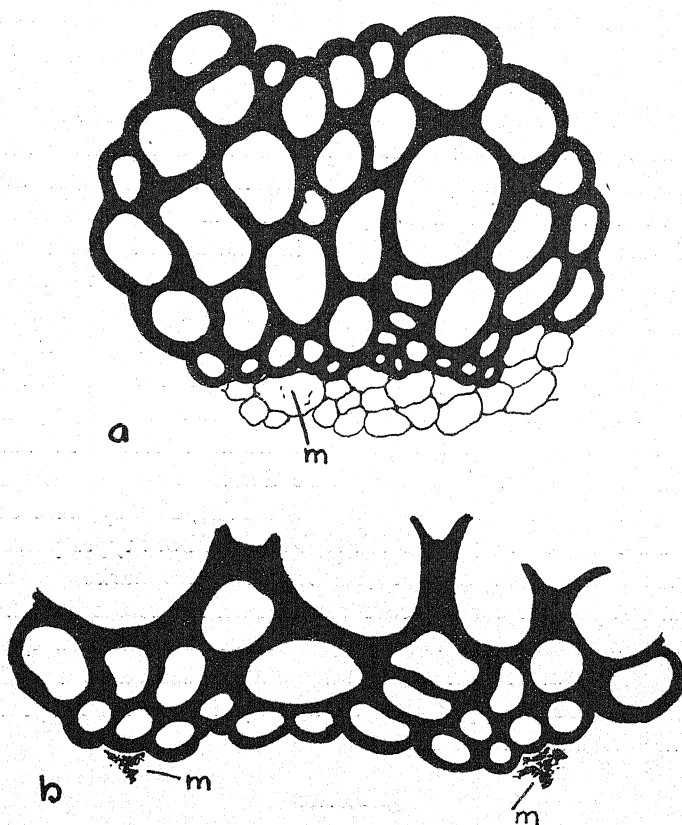
The cortex and epidermis of  $\alpha$  petioles present the same characters as those of  $\alpha$  stems.

iii.  $\beta$  petioles.—These also are circular in transverse section, and are

<sup>1</sup> Hick ('96), p. 10: 'the smaller segment seems to develop little or no xylem on its inner side.' In Pl. I, Fig. 5, Hick shows an  $\alpha$  stem and petiolar bundle from slide Q 101, Cash collect.; the protoxylem of the leaf-trace appears to be slightly immersed. In Figs. 3 and 4 (from Q 103 and Q 102) are shown two stages in the separation of a  $\beta$  petiole, in which the protoxylem is external.

<sup>2</sup> Neither Hick, Scott, nor Seward mentions the number of protoxylem groups in the petiolar bundle of *R. cylindrica*. Hick ('96), p. 11, says the petiolar bundle is 'semilunar in form with small elements on the convex side'. According to Scott ('08), p. 333, 'the nearly straight bundle has the protoxylem points all on the same side'. Seward ('10), p. 438, describes the leaf-traces as 'semilunar in section, with the protoxylem on the flatter side'. These three accounts indicate the variability of the petiolar vascular system.

smaller than the parent stems, having a diameter of about 1.3 mm. (Pl. XXVI, Fig. 7). The xylem strand, like that of the stem, is much reduced, measuring only about 0.15 mm.  $\times$  0.3 mm. Below the level of petiolar separation the trace is usually semilunar in transverse section (Pl. XXVI,



TEXT-FIG. 11. *a*, transverse section of a petiolar bundle, showing the adaxial distribution of the smaller elements, which seem to lie in two shallow grooves. At *m* there is an appearance similar to that figured in Pl. XXVII, Fig. 1, suggesting the breaking down of adaxial metaxylem elements. Note the general shape of the petiolar bundle, and the slight depression of the posterior margin.  $\times$  230. (From slide 1862, Williamson collect.) *b*, the anterior margin of a similar petiolar bundle, in which the protoxylem seems to be aggregated into two blunt points. At *m* the cells appear somewhat broken down.  $\times$  400. (From slide 1861, Williamson collect.) N.B. It is impossible definitely to refer this petiole to *R. cylindrica*, although its appearance suggests that it belongs to this species.

Fig. 9, *lt*; Pl. XXVII, Fig. 2); at a somewhat higher level it is bluntly triangular, the protoxylem being concentrated at the apex of the triangle. The monarch condition is maintained in  $\beta$  petioles, and no adaxial metaxylem is formed (Pl. XXVI, Figs. 7 and 9; Pl. XXVII, Fig. 2; Text-fig. 8), so that the protoxylem is always external.

The cortex of  $\beta$  petioles is wide in proportion to the size of the xylem

strand, and is similar to that of  $\beta$  stems. The outer layers are indefinite in structure.

iv. *Histology*.—In both  $\alpha$  and  $\beta$  petioles the details of cell structure follow very closely those described for the corresponding stems. No authentic longitudinal sections have been observed, but oblique sections show the scalariform and reticulate pitting of the tracheidal walls (see also a transverse section, Pl. XXVII, Fig. 1, *pt*). The protoxylem elements, as in the stems, are apparently scalariform. In the case of  $\beta$  petioles the metaxylem elements are frequently imperfectly lignified (Pl. XXVII, Fig. 2).

In some specimens the xylem strand does not occupy the centre of the stele, but is situated abaxially; it is probably this fact which caused Hick<sup>1</sup> to doubt whether the phloem completely surrounds the xylem. In such cases it is usually impossible to determine the structure of the external stelar tissues, on account of the crushing which they have undergone. In favourably preserved examples of  $\alpha$  petioles, however, the phloem appears to be continuous round the xylem, and to consist partly of narrow cells (Pl. XXVII, Fig. 1) and partly of larger cells suggestive of sieve-tubes. In  $\beta$  petioles the xylem almost completely fills the area enclosed by the rather ill-defined endodermis (Pl. XXVI, Fig. 7), and the nature of the small cells which immediately surround it cannot be determined. A pericycle may sometimes be recognized in  $\alpha$  petioles (Pl. XXVII, Fig. 1); and the endodermis (Pl. XXVI, Fig. 7; Pl. XXVII, Fig. 1) of both types possesses the same characters as that of the stems, although to a less marked degree. The contents of the petiolar cortical cells also are similar to those of the stems.

v. *Branching*.—In several cases petioles of *R. cylindrica* type, associated with stems of *R. cylindrica*, have been observed to contain small lateral traces; these are apparently produced by the division of a protoxylem group of the original petiolar bundle.

### 3. *Roots*.

i. *Development*.—Associated with the stems of *R. cylindrica* are numerous small roots, diarch and typically fern-like; similar structures may also be observed in various stages, arising endogenously from both  $\alpha$  and  $\beta$  stems<sup>2</sup> (Pl. XXVII, Fig. 4; Pl. XXVI, Fig. 8). They are scattered at fairly infrequent intervals, typically occurring singly in association with a petiole or branch<sup>3</sup> (Pl. XXVI, Figs. 3 and 8; Text-fig. 9); at times, however, two roots, at different stages, may be seen in the same transverse section of a stem (Pl. XXVII, Fig. 4).

<sup>1</sup> l. c., p. 11.

<sup>2</sup> Cf. Williamson ('78), Pl. 24, Fig. 87; Hick ('96), p. 11, and Pl. I, Fig. 2 (from the same specimen in Q 105, Cash collect., as Pl. XXVI, Fig. 8, of the present account).

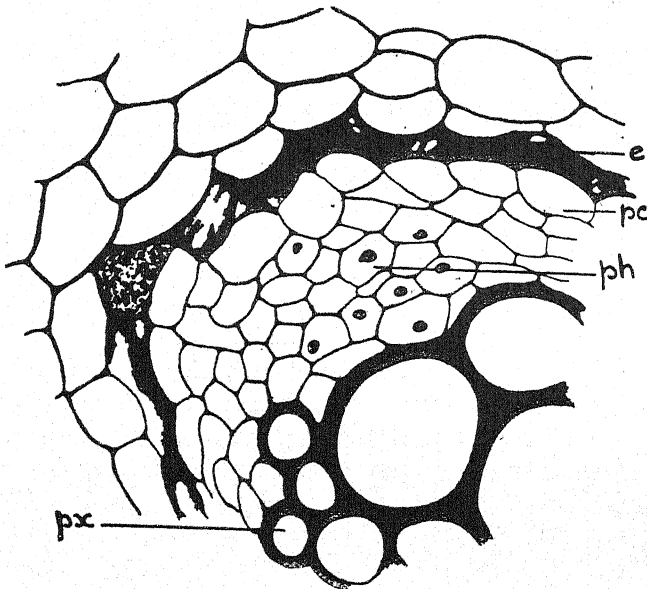
<sup>3</sup> Cf. Lachmann, J. P.: Contributions à l'histoire naturelle de la racine des Fougères. Ann. Soc. Bot. Lyon, Sér. A, No. 116, 1889. See p. 169. The distribution of the roots in *R. cylindrica* is not, according to Lachmann's conclusions, of very primitive type.



The vascular supply of each root is connected with a group of rather small tracheides occurring at the periphery of the stem xylem<sup>1</sup>; the details of root-formation are, however, difficult to observe.

The passage of the roots through the cortex is variable; it may be horizontal (Pl. XXVI, Fig. 3), or more or less vertical (Pl. XXVI, Fig. 8).

ii. *Anatomy and Histology*.—The roots of  $\alpha$  and  $\beta$  types are similar in structure; they vary considerably in size, an average diameter being 0.6 mm.; and they possess a diarch xylem-plate consisting of a few tracheides (Pl. XXVII, Fig. 3), all of which appear to be typically scalariform, although here



TEXT-FIG. 12. A portion of a well-preserved root, showing in transverse section the phloem-cells (*ph*), and the endodermis (*e*), the darkened cell-contents of which have here and there a pitted appearance; *px*, protoxylem; *pc*, pericycle.  $\times 400$ . (From slide K 21 m, University College, London.)

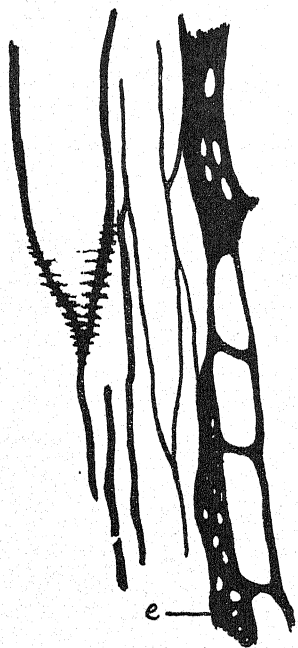
and there are indications of spiral thickening in the case of the protoxylem elements. The phloem is usually not well preserved; in a few cases, however, it may be recognized as a group of small thin-walled cells on either side of the xylem plate, alternating with its poles (Text-fig. 12). There appears to be a little conjunctive parenchyma, and occasionally a pericycle may be observed underlying the endodermis (Text-fig. 12). The cells of the pericycle are rather irregularly arranged, sometimes alternating with those of the endodermis, at other times being opposite to them. The endodermis is strongly marked, particularly in the older roots (Pl. XXVII, Fig. 3); it has the same characters as that of the stems and petioles, the pitted appearance often being very pronounced (Pl. XXVII, Fig. 3; Text-figs. 12 and 13) in both transverse and longitudinal sections

<sup>1</sup> Cf. Scott ('08), p. 329; and Seward ('10), p. 438.

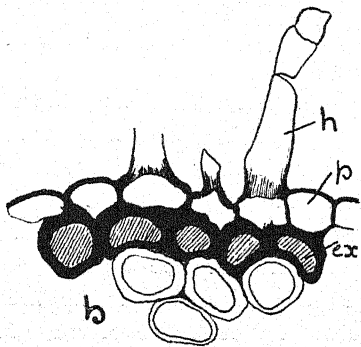
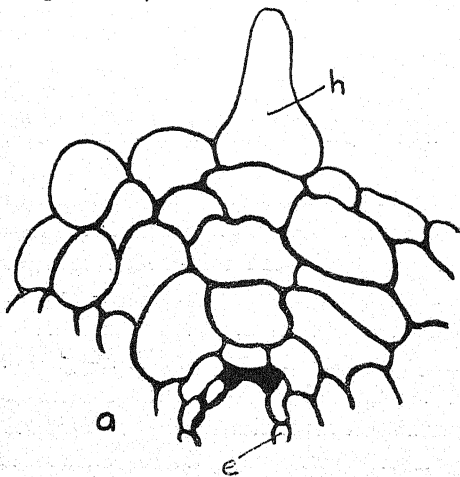
of the roots. In the younger examples, the endodermal cell-walls are firm and dark-coloured, but there is little or no blackening of the contents (Text-fig. 14, *a*). In one or two instances the cells of the endodermis in the neighbourhood of the xylem poles remain unblackened (Pl. XXVII, Fig. 3, *a*), as if their contents had been originally less dense. It is possible that these cells may have functioned as 'passage cells'.<sup>1</sup>

The cortical cells are regularly, and more or less concentrically, arranged in the innermost layers; they tend to become more irregular, and also slightly thicker-walled, towards the periphery of the root. There are apparently no intercellular spaces. The contents of the cortical cells are occasionally preserved, presenting similar characters to those described in the case of the stem, though they are much less dense.

In the majority of cases the outermost cortical layer, consisting of thick-walled darkened cells, forms the external covering, or exodermis, of the root. In one or two well-preserved examples, however, the outer piliferous layer is still present, overlying the



TEXT-FIG. 13. Part of an oblique longitudinal section of a root, showing the pitted appearance of the endodermis (*e*).  $\times 400$ . (From slide K 21 n, University College, London.)



TEXT-FIG. 14. *a*, a portion of a very young root showing the outgrowth of an external cell as a root-hair, *h*. *e*, endodermis of which most of the cells have unblackened contents.  $\times 400$ . (From slide K 21 i, University College, London.) *b*, a portion of a root in which the piliferous layer, *p*, and several root hairs, *h*, are shown; *ex*, exodermis.  $\times 230$ . (From slide Q. 108, Cash collect.)

<sup>1</sup> Haberlandt, G.: *Physiological Plant Anatomy*. English Edition, 1914. See pp. 370 and 372.

darkened outer cells of the cortex. It consists of regular thin-walled elements, which here and there have grown out to form root-hairs (Text-fig. 14, *b*). In another case root-hair production is shown in a very young root, the outer cortical cells of which are still unthickened.<sup>1</sup>

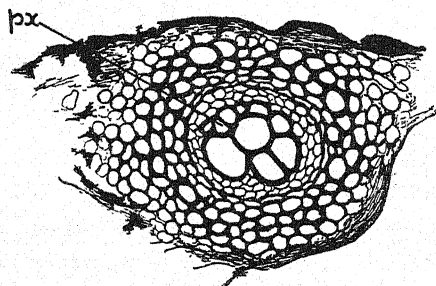
iii. *Branching*.—Occasionally lateral roots may be seen arising endogenously opposite to the protoxylem groups of the main root; the process, however, is too indistinct for detailed observation.

#### IV. ORGANS IN ASSOCIATION WITH *R. cylindrica*.

In fairly constant association with the stems of *Rachiopteris cylindrica* are 'axes' of varying sizes,<sup>2</sup> and detached sporangia similar in type to those associated with *Botryopteris ramosa*, *B. hirsuta*,<sup>3</sup> and *B. antiqua*.<sup>4</sup> Although there is usually no evidence of their identity with *R. cylindrica* beyond that of association, this, and their suggestiveness when compared with similar structures referred to allied species,<sup>5</sup> entitle them to a brief description.

##### 1. 'Axes' of various orders.

In several cases these detached 'axes' possess a monarch xylem-strand, similar to, but more robust than, the strand of  $\beta$  petioles (Text-fig. 15).



TEXT-FIG. 15. A monarch 'axis', associated with *R. cylindrica*. *px.*, protoxylem.  $\times 80$ . (From slide K 21 *d*, University College, London.)

The similarity of the cortex to that of  $\alpha$  organs suggests that these axes are branches of the primary  $\alpha$  petioles.<sup>6</sup>

<sup>1</sup> So far as the writer is aware, this is the first record of undoubted root-hairs in a Coal Measure plant.

<sup>2</sup> Williamson ('78) mentions (p. 351), and figures (Pl. 24, Figs. 81-3), axes much smaller than the main stems of *R. cylindrica*, and is inclined to regard them as branches of this plant.

<sup>3</sup> Scott ('08), pp. 332, 333; Fig. 125.

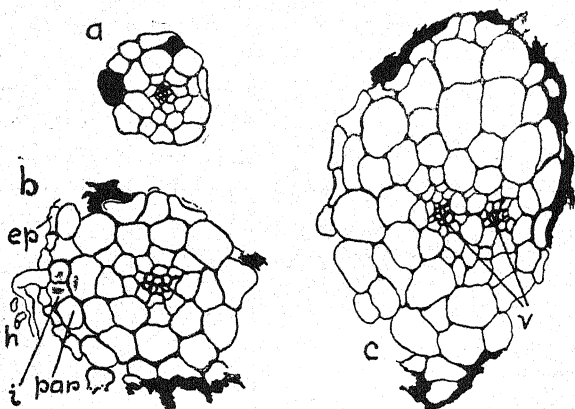
<sup>4</sup> Scott, D. H.: Sporangia attributed to *Botryopteris antiqua*, Kidston. Ann. of Bot., vol. 24, 1910, p. 819. See pp. 819 and 820.

Pelourde, F.: Observations sur quelques végétaux fossiles de l'Autunois. Ann. des Sci. Nat. (Bot.), sér. ix, vol. 11, 1910, p. 361. See p. 367, Fig. 6.

<sup>5</sup> See Section V. 2, p. 558.

<sup>6</sup> See p. 548.

Numerous examples occur of small axes in which the stele is reduced to a few tracheides surrounded by small thin-walled cells; there is no definite bundle-sheath (Text-fig. 16). The cells of the cortex are large, thin-walled, and irregular in shape. Usually the outermost cells are much crushed, or are entirely unrepresented; in a few instances, however, they may be seen to form a very loose tissue (Text-fig. 16, *b*), perhaps comparable with the assimilatory layer in the axes of *Stauropteris oldhamia*.<sup>1</sup> The epidermis consists of narrow thin-walled cells which may produce hairs (Text-fig. 16, *b*).



TEXT-FIG. 16. *a*, *b*, and *c*, transverse sections of small axes associated with *R. cylindrica*. In *b*, the outer cell-layers are shown at one point; *ep.*, epidermis, *h.*, hair, *par.*, parenchymatous cells, separated by intercellular spaces, *i.* In *c*, the vascular strand has divided into two equal groups, *v.*  $\times 80$ . (*a*, from slide K 21 *d*; *b*, from K 21 *e*; *c*, from K 21 *a*, University College, London.)

Dichotomously branching examples of these axes have been observed in both transverse (Text-fig. 16, *c*) and longitudinal section. The size of the axes is very variable (Text-fig. 16, *a*, *b*, and *c*), and is difficult to determine, owing to the crushing and removal of the outer cells; the largest seem to be somewhat smaller than the monarch 'axes' described above.

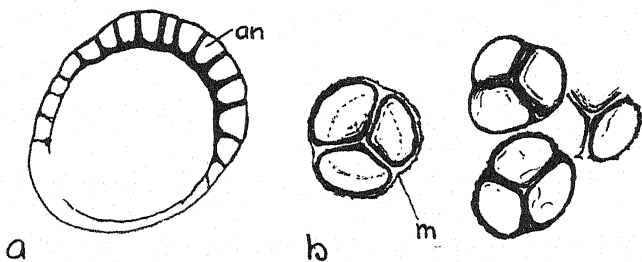
No gradations between these axes and either  $\alpha$  or  $\beta$  organs of *R. cylindrica* have been observed.

## 2. Sporangia.

The sporangia which occur in association with *R. cylindrica* are rounded or oval in section; the example shown in Text-fig. 17, *a*, measures  $325\mu$  by  $400\mu$ . The sporangium wall consists of a single layer of cells which become enlarged locally, forming a pluriseriate annulus (Text-fig. 17, *a*); the inner and lateral walls of the annulus cells are usually somewhat thickened.

<sup>1</sup> Scott ('05), p. 115.

The sporangia contain numerous tetrahedral spores, about  $25\mu$  in diameter; frequently the triradiate mark may be seen on the spore-coat. In the case of a young sporangium, spore-tetrads, some still enclosed in the mother-cell membrane, are well preserved (Text-fig. 17, *b*).



TEXT-FIG. 17. *a*, a section of a sporangium associated with *R. cylindrica*; *an.*, 'annulus' cells.  $\times 80$ . (From slide K 21 n, University College, London.) *b*, tetrad groups of spores in a young sporangium associated with *R. cylindrica*. *m.*, indications of the mother-cell membrane. In the left-hand group, the dotted line shows the position of the fourth spore, seen at a lower focus.  $\times 400$ . (From K 21 e, University College, London.)

## V. GENERAL DISCUSSION.

### 1. *The Significance of the Occurrence of $\alpha$ and $\beta$ types.*

The occurrence of the  $\alpha$  and  $\beta$  types of stems and petioles described above suggests three possibilities, namely, that they represent:

- i. different regions of the same plant;
- ii. two distinct though closely allied species;
- iii. habitat forms of a single species.

In favour of the first consideration it may be urged that the differences of structure between the two forms are not so great as those existing between different levels of growth in a single plant of *Psilotum*,<sup>1</sup> or of *Hottonia*.<sup>2</sup> But, although the two forms constantly occur side by side, no transition from one to the other has been observed amongst the numerous specimens examined.

With regard to the second suggestion, the points of similarity in structure and behaviour of  $\alpha$  and  $\beta$  stems and petioles are too numerous to warrant a multiplication of species names.

The available evidence tends to support the third possibility, indicating that the occurrence of  $\alpha$  and  $\beta$  types has a bearing upon the 'autecology' of *Rachiopteris cylindrica*.

The reduction of the xylem strand and its concentration, as exemplified by the presence of only one protoxylem group; the relatively wide cortex; the production of air-spaces; and the absence of mechanical tissue—features which characterize both stems and petioles of the  $\beta$  type, as compared with

<sup>1</sup> Williamson ('78), p. 351.

<sup>2</sup> Prankerd, T. L.: On the Structure and Biology of the genus *Hottonia*. Ann. of Bot., vol. 25, 1911, p. 253.

those of the *a* type—are modifications of structure which find their parallel among water-dwelling forms of recent plants, where they are too well known to require a detailed reference.<sup>1</sup>

It is therefore suggested that the differences between *a* and *β* types of *R. cylindrica* have been caused by the influence of water upon *β* individuals.<sup>2</sup>

With regard to the general habit of *R. cylindrica*, the presence of an outer cortical zone of thin-walled cells, which may very possibly be comparable with the assimilatory tissue of *Psilotum*, indicates that in the *a* type, which shows the characters of a land plant, the stems were exposed, and not of the nature of underground rhizomes. They may either have grown along the surface of the ground, rooting at the nodes; or, as their radial organization, lax habit, and fairly large petioles would suggest, they may have been semi-erect, supporting themselves upon other vegetation.

It must be remembered that any suggestions with regard to the ecological aspects of Coal Measure plants are necessarily very tentative; there is, however, some support for the view that the apparently straggling plants of *Rachiopteris cylindrica* grew at the edge of swamps or still lagoons.<sup>3</sup> The evidence drawn from the comparative structure of *a* and *β* types, and from their constant association with one another, points to the conclusion that the plasticity of the species—which is not necessarily connected with primitiveness<sup>4</sup>—allowed different individuals to exist side by side, some above, and others below, the water level. These individuals respectively constitute the *a* and *β* types, which may be regarded as habitat forms, or 'ecads' of the species.<sup>5</sup>

## 2. *The Relationships of R. cylindrica.*

Williamson,<sup>6</sup> in his original description of the organs referred to *Rachiopteris cylindrica*, mentioned the possibilities that they might be

<sup>1</sup> Cf. Schimper, A. F. W.: *Pflanzengeographie auf physiologischer Grundlage*, 1898.

Land and water forms of *Cardamine pratensis* (p. 26, Fig. 29) and of *Callitriche stagnalis* (p. 29, Fig. 32) show the same modifications of structure as *a* and *β* forms of *R. cylindrica*.

<sup>2</sup> The modifications are those caused by fairly still, rather than by strongly-flowing water; cf. Schimper ('98), p. 27.

If these conclusions be tenable, it is interesting to note the presence of hairs in both forms, for as Dombols (*Einfluss der geringeren oder grösseren Feuchtigkeit der Standorte der Pflanzen auf deren Behaarung*. Inaug.-Diss., Saarbrücken, 1887) has shown, these structures tend to be reduced or absent under the influence of moisture. A similar instance to that of *R. cylindrica*, however, is found in *Hottomia palustris*, where glandular hairs are present on the surface of the aquatic rhizomes and leaves, as well as on the land plants and aerial shoots.

<sup>3</sup> Cf. p. 532; and foot-note 2. The excellent preservation of the specimens, and the type of structural modification of *β* specimens, indicate the presence of fairly still water.

<sup>4</sup> Cf. the case of *Polygonum amphibium*.

<sup>5</sup> Clements, F. E.: *Research Methods in Ecology*. 1905. See pp. 148 and 316.

<sup>6</sup> Blackman, F. F., and Tansley, A. G.: *Ecology in its physiological and phytotopographical Aspects*. New Phyt., vol. 4, 1905, pp. 199 and 232. See p. 253.

<sup>6</sup> l. c., p. 351.

stems or roots of a Fern, or that they might represent some dwarf Lycopod type; that he personally regarded them as parts of a Fern is indicated by their inclusion in the provisional genus *Rachiopteris*.<sup>1</sup> In 1896 Hick<sup>2</sup> also inclined towards this opinion, which is now accepted without question.

Certain similarities between *R. cylindrica*, *R. ramosa*,<sup>3</sup> and *R. hirsuta*<sup>4</sup> suggest that these Lower Coal Measure species are closely allied; and since Scott<sup>5</sup> has included the two latter in Renault's genus, *Botryopteris*<sup>6</sup>—instituted for French Permo-Carboniferous types, of which *B. forensis* is the most completely known—several writers<sup>7</sup> consider that *R. cylindrica* should also be added. Scott,<sup>8</sup> however, while admitting a relationship between *R. cylindrica* and *Botryopteris* spp., retains for the present its original name, and suggests that it may be preferable to institute a new genus for its reception on account of its different habit.

A consideration of structural details indicates that *Botryopteris antiqua*, a species occurring in the Calciferous Sandstone of Pettycur,<sup>9</sup> and also, apparently, in the Culm of Esnost, near Autun,<sup>10</sup> is, of known types, the most nearly allied to *Rachiopteris cylindrica*, at least so far as the behaviour of the foliar trace<sup>11</sup> is concerned. There is also evidence that, in this respect, *R. cylindrica* represents a transition stage between *B. antiqua* on the one hand and *B. ramosa* and *B. hirsuta* on the other.

According to Gordon<sup>12</sup> and to Benson,<sup>13</sup> the petiolar trace in *B. antiqua*

<sup>1</sup> Williamson, W. C.: On the Organization of the Fossil Plants of the Coal Measures. VI. Ferns. Phil. Trans. Roy. Soc., B, vol. 164, 1874, p. 675. See p. 677.

<sup>2</sup> l. c., p. 14.

<sup>3</sup> Williamson, W. C.: On the Organization of the Fossil Plants of the Coal Measures. XVIII. Phil. Trans. Roy. Soc., B, vol. 182, 1891, p. 255. See p. 261.

<sup>4</sup> Williamson, W. C.: On the Organization of the Fossil Plants of the Coal Measures. XV. Phil. Trans. Roy. Soc., B, vol. 180, 1889, p. 155. See p. 161.

<sup>5</sup> Scott, D. H.: On an English *Botryopteris*. Report of the British Association Meeting at Bristol, 1898, Section K, p. 1050.

Scott, D. H.: Studies in Fossil Botany. First edition, 1900, p. 291.

<sup>6</sup> Renault, B.: Recherches sur les végétaux silicifiés d'Autun et de St.-Étienne. Étude du genre *Botryopteris*. Ann. des Sci. Nat. (Bot.), sér. vi, t. 1, 1875, p. 220.

(See also Cours de Botanique Fossile, t. 3, 1883, p. 104; and Bassin Houiller et Permien d'Autun et d'Épinac: Flore Fossile, Pt. II, 1896, p. 33.)

<sup>7</sup> Browne ('08), p. 57; Seward ('10), pp. 438-40. See also Tansley ('08), p. 14.

<sup>8</sup> Scott ('08), pp. 333 and 335.

<sup>9</sup> Kidston ('08).

Benson, M.: New Observations on *Botryopteris antiqua*, Kidston. Ann. of Bot., vol. 25, 1911, p. 1045.

<sup>10</sup> Bertrand, C. E., and Cornaille, F.: Les caractéristiques de la trace foliaire botryoptéridienne. Comptes rendus des Séances de l'Académie des Sciences, t. 150, 1910, p. 1019. See p. 1022. Pelourde ('10), p. 364.

Bertrand, P.: L'étude anatomique des Fougères anciennes, et les problèmes qu'elle soulève. Progressus Rei Botanicae, vol. 4, 1912, p. 182. See p. 232.

<sup>11</sup> In a study of relationships the evidence of the foliar trace appears to be of considerable value. See Bertrand ('12).

<sup>12</sup> l. c. ('10), p. 400.

<sup>13</sup> l. c., p. 1047.

has at its lowest levels a single immersed pole near its anterior margin. As the trace passes out, the protoxylem, which either remains single or is duplicated, becomes external, its elements lining one or two shallow adaxial grooves. Sometimes the protoxylem elements appear to be distributed on the anterior margin of the trace.<sup>1</sup>

In the case of *R. cylindrica* the foliar trace possesses at its lowest level a single external pole; a little higher in the trace, the presence of a few adaxial metaxylem elements, in some instances, indicates a relic of an ancestral structure, such as that realized in *B. antiqua*. The protoxylem is, at still higher levels, more or less external in all cases, its behaviour and arrangement in the typical  $\alpha$  petioles being very similar to that described for *B. antiqua*. Sometimes, however, when the protoxylem groups appear to form two minute points, there is a hint of progression towards the condition seen in the tridentate <sup>2</sup> petioles of *Botryopteris ramosa* and *B. hirsuta*. In these species, the petiolar vascular bundle is typically triarch, the protoxylem elements being aggregated into points of very varying prominence in different specimens. The three groups result from the division of the single pole of the leaf-trace.

Miss Benson<sup>3</sup> explains the increase in the number of poles from one to three in the evolution of the botryopteridean trace, as being due to the arrest of branching at an early stage; this increase seems to be accompanied by the gradual protrusion of the protoxylem groups.<sup>4</sup> In accordance with this view, it is suggested that *R. cylindrica* represents an intermediate, though not very advanced, stage in the series, for while protrusion of the protoxylem is indicated in some specimens, its arrangement is usually more reminiscent of that in *B. antiqua*.

According to Paul Bertrand's earlier work,<sup>5</sup> the botryopteridean trace, as represented by that of *B. forensis*, may be derived from the reduction of an ancestral bipolar form. As Scott<sup>6</sup> notes, this author does not take into account the trace in related and older types; while Miss Benson<sup>7</sup> shows that if Bertrand's view be accepted, the early occurrence of a monarch trace, in *B. antiqua*, indicates a process of simplification within the series—an indication with which the triarch petiolar bundles of later species are not in harmony. It seems more reasonable to regard at least *B. antiqua*,

<sup>1</sup> Kidston ('08), p. 363; Pelourde ('10), p. 365, Fig. 2.

<sup>2</sup> Felix, J.: Untersuchungen über den inneren Bau westfälischer Carbonpflanzen. Abhandl. Kön. Preuss. geol. Landesanst., Bd. 7, 1886, p. [153]. See p. [164], and Taf. I, Fig. 2.

Scott ('98), p. 1050.

In  $\beta$  petioles, the single pole typically forms a distinct point.

<sup>3</sup> l. c., p. 1051.

<sup>4</sup> *ibid.*, Text-fig. 2, p. 1051.

<sup>5</sup> l. c. ('09), p. 238.

<sup>6</sup> Scott, D. H.: Review of Dr. P. Bertrand's work, *Études sur la fronde des Zygoptéridées* (Lille, 1909). New Phyt., vol. 8, 1909, p. 266. See p. 271.

<sup>7</sup> l. c., p. 1053. See also Bertrand's general agreement with Miss Benson's criticism, ('12), p. 233.



*R. cylindrica*, and *B. ramosa* and *hirsuta* as forming a series in which the foliar traces show progression from a simple structure in the oldest species to a more complex development in the later species; for, as Bertrand<sup>1</sup> now admits, the simple trace of *B. antiqua* may readily be derived from a basal, generalized form—a rounded or oval mass of wood having a single central pole—by a slight anterior displacement of the protoxylem. According to this view, the foliar traces of the Botryopterideae, Osmundaceae, Psaronieae, and Zygopterideae may be referred to a common ancestral type.<sup>2</sup>

The branching of the petiolar bundles in *B. antiqua*,<sup>3</sup> *B. ramosa*, and *B. hirsuta* is lateral, and there are indications that it is of the same type in *R. cylindrica*. The secondary traces are usually smaller than the parent strand, and their protoxylem is apparently provided by the division of one of the petiolar groups. In the case of the triarch petioles of *B. ramosa* and *B. hirsuta*, the central protoxylem group is not concerned in the production of branch traces.

With regard to stem structure, *R. cylindrica* is essentially very similar to the three British species of *Botryopteris*. In each case the stem is protostelic, the differences depending chiefly upon the varying position of the protoxylem groups. It is not, however, possible to trace an evolutionary series like that exhibited by the foliar bundles.

In *B. antiqua* the position of the protoxylem is variable and indefinite<sup>4</sup>; many scattered peripheral elements occur in the root-bearing zone of the stem. These are no doubt comparable with the small elements giving rise to roots in the other species. In the leaf-bearing zone, there are only one or two mesarch groups; the same number occurs in *B. ramosa*, where they are, however, placed towards the centre of the xylem strand; *B. hirsuta* apparently presents a similar condition. It has been shown that the vascular strand of *R. cylindrica* may possess a single endarch group, or from two to five groups in a mesarch position, the chief distinction between this and the other species consisting in the slight and varying differentiation of the internal xylem in typical specimens. Whether this differentiation is indicative of a higher or lower development of the vascular strand is open to much discussion; its theoretical significance will be discussed in the next section.<sup>5</sup>

The similarity in general stem structure in these species extends to the pitting of the tracheides, which is of scalariform and reticulate type. Scalariform pitting seems to be predominant in *B. antiqua*, and reticulate pitting in *B. ramosa* and *B. hirsuta*; whether there is any significance in

<sup>1</sup> l. c. ('12), p. 233.

<sup>2</sup> Bertrand ('12), p. 299.

Kidston, R., and Gwynne-Vaughan, D. T.: On the Fossil Osmundaceae. Pt. I. Trans. Roy. Soc. Edin., vol. 45, Pt. III, 1907, p. 759. See pp. 777 and 778.

Gordon ('11), p. 733.

<sup>3</sup> Benson ('11), p. 1048.

<sup>4</sup> *ibid.*, p. 1046.

<sup>5</sup> p. 561.

this fact<sup>1</sup> is uncertain, for in *R. cylindrica* it is evident that the distribution of the two types depends upon the width of the tracheide walls.<sup>2</sup>

There is thus considerable agreement in the organization of the four species, and in view of this fact it is interesting to note that the associated sporangia in each case are of similar type.

It is inadvisable to draw any conclusions from the apparent absence of a leaf of ordinary Fern type within this group. In *B. ramosa* there are indications that the frond was much dissected, as in *Stauropteris* and members of the Zygopterideae; while the presence of small axes associated with *R. cylindrica* suggests incomplete foliar development in this species also—a suggestion which is supported by the probable assimilatory nature of the outer cortex of the stem and petiole. Smaller axes, again, occur in association with the petioles of *B. antiqua*, but it is as yet impossible to say what the exact condition of foliar development may have been in these plants; and in any case, similarities or differences may have been due to the influences of environment rather than to degree of relationship.

The four species differ to a certain extent in habit. *B. antiqua* is considered by Kidston<sup>3</sup> to have been a scrambling plant requiring support for its large leaves, some of which were accompanied by sheathing aplebiae.<sup>4</sup> Seward<sup>5</sup> suggests that the slender plants of *B. ramosa*, with their much branched leaves, were epiphytic in habit; while *B. hirsuta* seems to have been similar, though with larger and less crowded leaves. There are indications that *R. cylindrica* was an 'amphibious' plant; its dichotomously branched stems were of lax habit and bore few leaves, the inadequacy of which is suggested by the development of apparent cauline assimilatory tissue.

These differences, however, are not greater than are to be found amongst closely-related types—even within a single genus—at the present day. The diversity of habit, and of habitat, amongst the species of *Polygonum* may be mentioned as an example.

It may be concluded that *B. antiqua*, *R. cylindrica*, *B. ramosa*, and *B. hirsuta* form a group of closely-related species, showing, at least in the behaviour of the foliar trace, a gradual transition from a simple to a more complex structure.

*Botryopteris forensis*, as representative of the French Permo-Carboniferous species, seems to stand a little apart from the group of older British species.

<sup>1</sup> Stopes, M. C.: A New Fern from the Coal Measures: *Tubicaulis Sutcliffii*, spec. nov. Mem. and Proc. Manchester Lit. and Phil. Soc., vol. 50, Pt. III, 1906. See p. 24.

Gordon ('10), p. 400; Seward ('10), p. 436.

<sup>2</sup> Cf. Scott ('08), p. 326.

<sup>3</sup> l. c., p. 364.

<sup>4</sup> Benson ('11), pp. 1048 and 1049; Text-figs. 1 a and 1 b.

<sup>5</sup> l. c., p. 441.

With regard to the foliar trace, the exact details of its emission have not been described, but it is evident that the fully-formed petiolar bundle is much more complicated than that of the four species just considered. It is, in general shape, like an  $\omega$ , the arms of which point towards the centre of the stem, and it has been compared with the tridentate bundles of *B. ramosa* and *B. hirsuta*, where the three projections are much smaller.

In *B. forensis* the foliar bundle is described as consisting of an essential or 'principal' segment—the centre arm of the  $\omega$ ; and two accessory or 'receptive' segments—the two lateral arms.<sup>1</sup> These latter are believed by Bertrand to be secondary developments to compensate for the extreme reduction of the essential part of the trace and to provide the metaxylem of the branch-traces. The principal segment was originally considered to have been derived from a primitive bipolar trace, in which extreme contraction of the anterior surface had taken place<sup>2</sup>; more recently Bertrand<sup>3</sup> has admitted that the Botryopteridean trace may have been derived directly from a unipolar ancestor. In either case, however, its reduction was apparently such as to require the development of the receptive parts, and these were, according to Bertrand<sup>4</sup> already present in the older types *B. antiqua* and *B. ramosa*, although in a condensed state, forming one mass with the principal segment.

Branching phenomena certainly demonstrate that the centre arm of the  $\omega$  must be regarded as the essential segment. At the base of the petiole, it possesses, at its free extremity, two lateral, slightly-sunken poles,<sup>5</sup> which by their division provide the protoxylem strands of the branch traces.

The central arm of the  $\omega$  is thus, at this stage, a much more elaborate structure than the central point of a tridentate petiolar bundle. Higher in the leaf, however, it possesses only a single terminal protoxylem group, and according to Bertrand and Cornaille,<sup>6</sup> further reduction of the trace produces tridentate bundles, similar to those of *B. hirsuta*, which, as already mentioned, are also held to include principal and receptive segments in a condensed state. The ultimate traces of *B. forensis* are monarch, but these cannot be compared with the monarch phase of the earlier species, for they are said to consist only of the two receptive segments, the principal segment not being represented at this high level; the monarch phase of the British species, on the other hand, must include the principal segment.<sup>7</sup>

The significance of these facts is doubtful; for if *B. forensis*, *B. ramosa*, and *B. hirsuta* belong to the same series, and if the simpler tridentate traces are representative of a reduction phase, then the geologically older

<sup>1</sup> Bertrand ('09), p. 238; Bertrand and Cornaille ('10), p. 1019; Bertrand ('12), pp. 230 and 231.

<sup>2</sup> Bertrand ('09), p. 238.

<sup>3</sup> l. c. ('12), p. 233.

<sup>4</sup> l. c. ('12), p. 232.

<sup>5</sup> Bertrand ('12), p. 231, Fig. 26.

<sup>6</sup> l. c., p. 1022.

<sup>7</sup> *ibid.*, p. 1022.

species of the genus realize this phase at a lower level of petiolar development—that is, at the *base* of the petiole instead of in an ultimate branch. They may thus be regarded as further from the ancestral type in this respect than *B. forensis*; also their simplicity cannot be due to primitiveness.

Again, since the traces of *B. antiqua* and *R. cylindrica* are clearly simpler members of the same series as the tridentate forms,<sup>1</sup> it may be argued that further simplicity is due to further reduction; if this be so, the oldest species of the series must possess the most reduced trace.

It has been shown, however, that the foliar traces of *B. antiqua*, *R. cylindrica*, and *B. ramosa* and *hirsuta* form a connected progressive series which may be derived from an ancestral type of trace; and, in the absence of advancing intermediate stages connecting the simple tridentate traces with that of *B. forensis*, it may be advisable to consider this species as being somewhat removed from the earlier types.

In stem structure also, *B. forensis* differs from the British types, for the solid protostele is definitely exarch.<sup>2</sup>

The plant is known to have possessed a leaf with thick, dichotomously branched veins and small fleshy pinnules—the only example of an ordinary leaf yet known amongst the species of *Botryopteris*.

The sporangia, again, seem to isolate *B. forensis* from the British species. They have been described as possessing a two-layered wall,<sup>3</sup> the inner layer being often distinguishable only as a thin membrane. According to Oliver,<sup>4</sup> some specimens referred to the provisional genus *Tracheotheca*<sup>5</sup> may perhaps have belonged to *B. forensis*; their walls are lined with a delicate tracheal layer unlike anything observed in the earlier species. Its presence, of course, may be merely the result of environmental influences.<sup>6</sup>

It is concluded that *Rachiopteris cylindrica* is allied to *Botryopteris antiqua*, *B. ramosa*, and *B. hirsuta*, the four species forming a group, with which *B. forensis* is not very closely related. This conclusion suggests that the desirability of including *Rachiopteris cylindrica* in the genus *Botryopteris* must be determined by the desirability of retaining there its three related types.

### 3. Theoretical Considerations.

*Rachiopteris cylindrica* presents several points of theoretical interest, two of which will be briefly discussed.

<sup>1</sup> Kidston ('08), p. 364.

<sup>2</sup> Renault ('83), p. 104; Seward ('10), p. 437.

<sup>3</sup> Renault ('96), pp. 53 and 54.

<sup>4</sup> Oliver, F. W.: A Vascular Sporangium. *New Phyt.*, vol. 1, 1902, p. 60.

<sup>5</sup> Oliver, F. W.: On the Structure and Affinities of *Stephanospermum*, Brongniart, a Genus of Fossil Gymnosperm Seeds. *Trans. Linn. Soc., Bot.*, vol. 6, 1904, p. 361. See foot-note, p. 395.

<sup>6</sup> Seward ('10), p. 443.

i. *The Primitiveness of the Stelar Condition.*

*R. cylindrica* has been described by Tansley<sup>1</sup> as possessing an endarch protostele, and as being therefore primitive in this respect. But it has been shown that the  $\alpha$  forms, at least, are not in the majority of cases typically endarch; they rather tend towards mesarchy,<sup>2</sup> and even, in some specimens, towards a differentiation of the xylem into inner and outer zones.

On the assumption of Tansley's theory—and there is much in favour of the primitiveness of endarchy amongst the ancestors of vascular plants as a whole<sup>3</sup>—the 'mesarch'  $\alpha$  stems are to be considered as having made a slight advance upon the primitive condition, particularly in those cases where there is differentiation of the internal wood.<sup>4</sup> True endarchy is met with in some branches of  $\alpha$  stems and in the  $\beta$  stems; its occurrence in *R. cylindrica*, however, cannot be considered as having any bearing upon the primitiveness of the vascular structure; for in  $\alpha$  stem branches it represents a derived and reduced condition, due probably to decrease of vigour, and in  $\beta$  types, if the argument of concentration under the influence of a water habitat be tenable, the same condition is again due to reduction.

From a study of the Fossil Osmundaceae, Kidston and Gwynne-Vaughan<sup>5</sup> conclude that the ancestral form of this group must have possessed an exarch protostele; and Lady Isabel Browne<sup>6</sup> is of the opinion that exarchy represents the primitive condition of the Pteridophyta as a whole. Bertrand,<sup>7</sup> however, considers that if the protostele is to be regarded as the original stelar type, its protoxylem groups were most likely slightly immersed.

According to any of the above views, it is evident that typical specimens of *Rachiopteris cylindrica*—apart from the cases where endarchy occurs as a state of reduction—show some divergence from the ancestral structure.

A consideration of Bertrand's theory of the 'étoile libéro-ligneuse',<sup>8</sup> or 'asterostele', suggests that such forms as the mesarch  $\alpha$  stems of *R. cylindrica* may be more primitive than truly endarch forms, since they may represent a stage in the condensation of an ancestral rayed structure like that of *Cladoxylon*; the endarch condition itself, according to Bertrand, denotes a very advanced state of condensation. It has certainly been shown that, in  $\alpha$  stem branches, endarchy results from the condensation of a dispersed condition of the protoxylem groups; as mentioned above, this may be due to a decrease of vigour. In  $\beta$  stems, endarchy is considered to be due to the action of environment, causing concentration of the vascular tissues. Since, in these cases, the occurrence of endarchy may be explained on physiological grounds, it can hardly be accorded any phylogenetic

<sup>1</sup> l. c., pp. 14 and 15.

<sup>4</sup> Gordon ('10), p. 400.

<sup>7</sup> l. c., ('12), p. 263.

<sup>2</sup> Browne ('08), p. 57.

<sup>5</sup> l. c., p. 777.

<sup>8</sup> Bertrand ('12), pp. 249–51.

<sup>3</sup> Tansley ('08), p. 15.

<sup>6</sup> l. c., p. 58.

significance. It is only possible to say that if the theory of the asterosteles be tenable, typical examples of *R. cylindrica* represent a stage much in advance of the primitive condition.

According to Lignier,<sup>1</sup> the primitive vascular system was a solid exarch xylem mass; during the course of evolution this underwent a process of dissection and of subsequent concentration which, to a certain extent, recalls the behaviour of Bertrand's asterosteles. Lignier's theory, again, would place *R. cylindrica* far from the original type.

It is therefore impossible to say with certainty whether the stele of *R. cylindrica* is more or less highly organized than those of related species; it is, however, reasonable to conclude that typical examples are not primitive according to any of the theories mentioned above.

## ii. The Homology of the Leaf.

A comparison of the methods of stem-branching and leaf-production in *R. cylindrica* provides evidence in favour of the view, suggested by Bower<sup>2</sup> in 1884, that stem and leaf are homologous branches of a primitively undifferentiated and dichotomous system. This view is now the basis of the hypothesis set forth by Potonié,<sup>3</sup> Hallier,<sup>4</sup> Lignier,<sup>5</sup> Tansley,<sup>6</sup> and Bertrand<sup>7</sup>; according to Tansley it 'carries with it the necessity of looking upon the branching away of the leaf-trace from the vascular system of the stem as in origin a separation of the vascular strand into branches of equivalent morphological status'.<sup>8</sup>

Stem-branching and leaf-production have been described in *R. cylindrica*, and it is evident that the two processes are essentially the same in origin. In branching, however, the completion of both branch steles is ensured by the formation of metaxylem elements below the actual level of their separation (see Pl. XXVI, Figs. 5 and 9; Text-fig. 7); in leaf-production, on the other hand, only the stem stele is completed in this way, for at the

<sup>1</sup> Lignier, O.: Organisation progressive du parcours des faisceaux libéro-ligneux dans le méristème des Phyllinées. Bull. Soc. Bot. de France, t. 58, 1911, p. 29.

Lignier, O.: Essai sur les transformations de la stèle primitive dans l'embranchement des Phyllinées. Bull. Soc. Bot. de France, t. 58, 1911, p. [87].

<sup>2</sup> Bower, F. O.: On the Comparative Morphology of the Leaf in the Vascular Cryptogams and Gymnosperms. Phil. Trans. Roy. Soc., B, vol. 175, 1884, p. 565. See p. 605. (Bower has now abandoned this theory.)

<sup>3</sup> Potonié, H.: Ein Blick in die Geschichte der botanischen Morphologie und der Pericaulomtheorie. 1903, p. 33.

See also Lehrbuch der Pflanzenpalaeontologie, 1899, pp. 156-9; and other references.

<sup>4</sup> Hallier, H.: Beiträge zur Morphogenie der Sporophylle und des Trophophylls in Beziehung zur Phylogenie der Kormophyten. Jahrb. der Hamburgischen wissenschaftlichen Anstalten, 19, 1901 (published 1902). See p. 45 and p. 104.

<sup>5</sup> Lignier, O.: Essai sur l'évolution morphologique du Règne végétal. Bull. Soc. Linn. de Normandie, sér. 6, vol. 3, 1908-9, p. 35. Reprinted, 1911. See other references also.

<sup>6</sup> l. c., p. 1 of reprint.

<sup>7</sup> l. c. ('09), pp. 260, 261; ('12), p. 278.

<sup>8</sup> l. c., p. 3 of reprint (cf. New. Phyt., vol. 6, 1907, p. 26).

place of separation the leaf-trace does not appear to possess any adaxial metaxylem (Pl. XXVI, Figs. 6 and 9; Text-figs. 8 and 9). A few elements may be developed higher in the petiolar trace, but at a slightly higher level still they tend to disappear again; their formation may be regarded as indicating an earlier condition similar to that seen in *Botryopteris antiqua*, in which some adaxial metaxylem is present at the level where the trace separates from the stem stele, and which is therefore still more suggestive of modified stem-branching.

This view of the origin of the leaf is further supported by the similar behaviour of the protoxylem in branching and in leaf formation. In  $\alpha$  types, the protoxylem group of both branch- and leaf-traces divides more or less definitely; in  $\beta$  types, no division normally takes place in either instance.

## VI. SUMMARY.

### 1. *Distribution and Horizon*, p. 532.

*Rachiopteris cylindrica* appears to be restricted to the Halifax-Huddersfield area, where it occurs in the nodules of the Halifax Hard Bed of Lower Coal Measure Age.

### 2. *Description*, p. 532.

The stems and their corresponding petioles may be referred to two types, described as  $\alpha$  and  $\beta$  respectively.

i.  $\alpha$  stems are characterized by a well-developed xylem strand exhibiting a marked tendency towards mesarch structure, with differentiation of the central elements; the inner and middle cortical areas have fairly thick-walled cells, while the outer cortex is composed of a few layers of thin-walled cells, suggestive of an assimilatory tissue.  $\alpha$  petioles also have well-developed xylem strands, frequently with distinct diarch structure; their cortex is like that of  $\alpha$  stems.

ii.  $\beta$  stems possess only a small monarch, centrarch xylem strand. The cortex is wide and composed of thin-walled cells; the middle area is more or less lacunar, and the outer layers of the stem seem to be of the same nature as those of  $\alpha$  stems. The corresponding petioles have also a wide cortex, and a reduced xylem strand which is always monarch.

### 3. *The Significance of the Occurrence of $\alpha$ and $\beta$ types*, p. 553.

It is probable that the differences of structure between the  $\alpha$  and  $\beta$  types throw some light on the autecology of *Rachiopteris cylindrica*, which, it is suggested, was amphibious,  $\alpha$  and  $\beta$  plants being respectively its land and water ecads.

### 4. *Relationships*, p. 554.

*R. cylindrica* seems to be closely allied to *Botryopteris antiqua*,

*B. ramosa*, and *B. hirsuta*. So far as the foliar trace is concerned, the four species form a progressive series from the relatively primitive *B. antiqua* to the tridentate types, *R. cylindrica* representing an intermediate term. *B. forensis* does not appear to be very nearly related to this group of British species.

### 5. *The Primitiveness of the Stelar Condition*, p. 561.

Typical steles of *R. cylindrica* show some divergence from the primitive condition, whether this is considered to be an endarch or an exarch proto-stele, or an asterosteale.

### 6. *The Homology of the Leaf*, p. 562.

The method of separation of the foliar trace in *R. cylindrica* affords support to the view that stem and leaf represent homologous branches of a primitively undifferentiated system.

UNIVERSITY COLLEGE,  
LONDON.  
May, 1915.

## EXPLANATION OF PLATES XXVI AND XXVII.

*px.* = protoxylem; *xi.* = inner wood; *xo.* = outer wood; *amx.* = adaxial metaxylem; *st.* = sieve-tubes; *pc.* = pericycle; *e.* = endodermis; *ic.* = inner cortex; *mc.* = middle cortex; *oc.* = outer cortex; *l.* = lacunae of middle cortex; *c.* = cavities of outer cortex; *rt.* = root-trace; *lt.* = leaf-trace; *b.* = branch stele; *par.* = parenchyma; *s.* = stem; *p.* = petiole.

### PLATE XXVI.

#### *Rachiopteris cylindrica*.

Fig. 1. A typical  $\alpha$  stem, showing the mesarch protoxylem groups, and the slight differentiation of the internal wood. Note the somewhat concentric inner layers of the cortex; the thin-walled crushed cells composing the outer cortex, and the cavities in this layer.  $\times 32$ . (From Q 531, Cash collection.)

Fig. 2. A centrarch  $\alpha$  stem.  $\times 27$ . (From K 21 d, University College, London.)

Fig. 3. A  $\beta$  stem, showing the small stele, with central protoxylem, and the wide cortex of thin-walled cells. At *r*, a root has just passed out; in the cortex is a small leaf-trace; at *l*, the lacunar structure of the middle cortex is shown.  $\times 27$ . (From K 21 d, University College, London.)

Fig. 4. A  $\beta$  stem, in which the middle cortex possesses large lacunae. The stele of this stem does not show the typical condition, as it possesses two protoxylem groups.  $\times 36$ . (From Q 103, Cash collection.)

Fig. 5. An  $\alpha$  stem in which the stele is preparing for dichotomy. The branch-stele is slightly smaller than the parent stele; it possesses two protoxylem groups and some adaxial metaxylem (cf. Fig. 6, in which the leaf-trace has no adaxial metaxylem).  $\times 40$ . (From K 21 n, University College, London.)

Fig. 6. An  $\alpha$  stem preparing for leaf-production. Separating parenchymatous cells are present immediately in front of the single protoxylem of the leaf-trace (cf. Fig. 5).  $\times 40$ . (From K 20 k, University College, London.)

Fig. 7. A later stage of the  $\beta$  stem and leaf-trace shown in Fig. 3; both stem and petiole possess a small stele, and wide cortex.  $\times 27$ . (From K 21 e, University College, London.)



Fig. 8. A  $\beta$  stem just above dichotomy of the stele; each branch has only one protoxylem group. A root  $r$  is passing through the cortex; the crushed outer cortex is indicated at several points.  $\times 32$ . (From Q 105, Cash collection.)

Fig. 9. A  $\beta$  stem preparing for dichotomy, immediately above the level of leaf-trace production. The middle cortex is much crushed.  $\times 58$ . (From K 217, University College, London.)

PLATE XXVII.

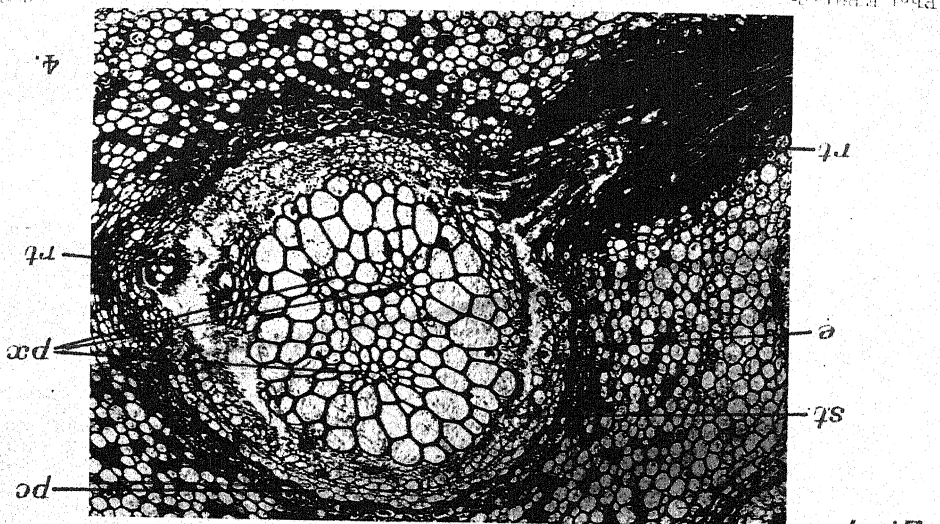
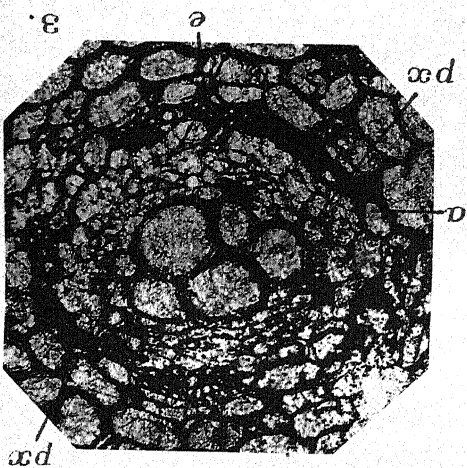
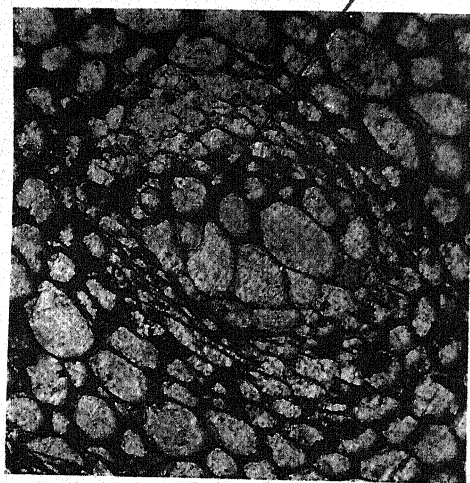
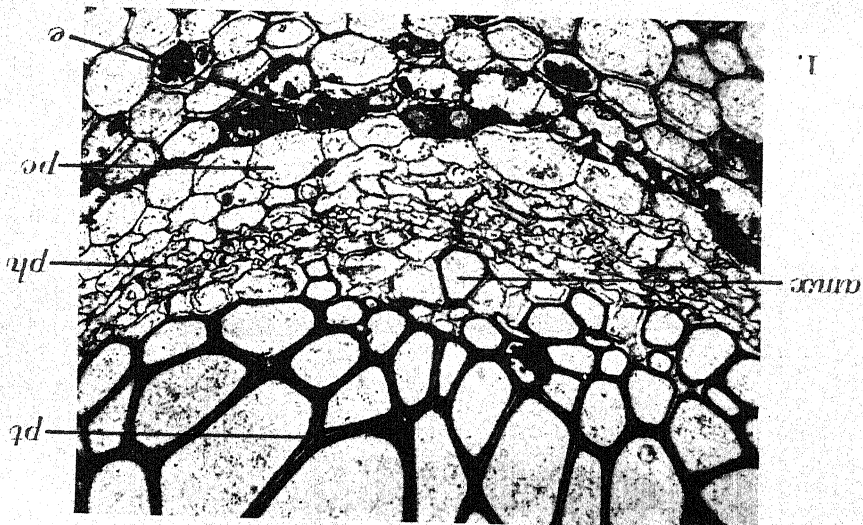
*Rachiopteris cylindrica*.

Fig. 1. The adaxial margin of the bundle in an  $\alpha$  petiole just above the separation of the latter from the stem. The anterior metaxylem appears to be breaking down, and the protoxylem is indefinite. Note the crushed phloem cells ( $ph$ ), and the transverse section of the pits ( $pt$ ) on the tracheide walls.  $\times 40$ . (From slide 302.6 in Dr. Gordon's collection.)

Fig. 2. A petiolar bundle passing through the cortex of a  $\beta$  stem. Note the single protoxylem group, and the imperfectly-lignified metaxylem.  $\times 200$ . (From K 217, University College, London.)

Fig. 3. The stele of a diarch root, showing the pitted appearance of the endodermis. Certain of the cells appear to be less resistant than the others; note particularly a cell  $a$  in the neighbourhood of one of the protoxylem groups.  $\times 200$ . (From K 217, University College, London.)

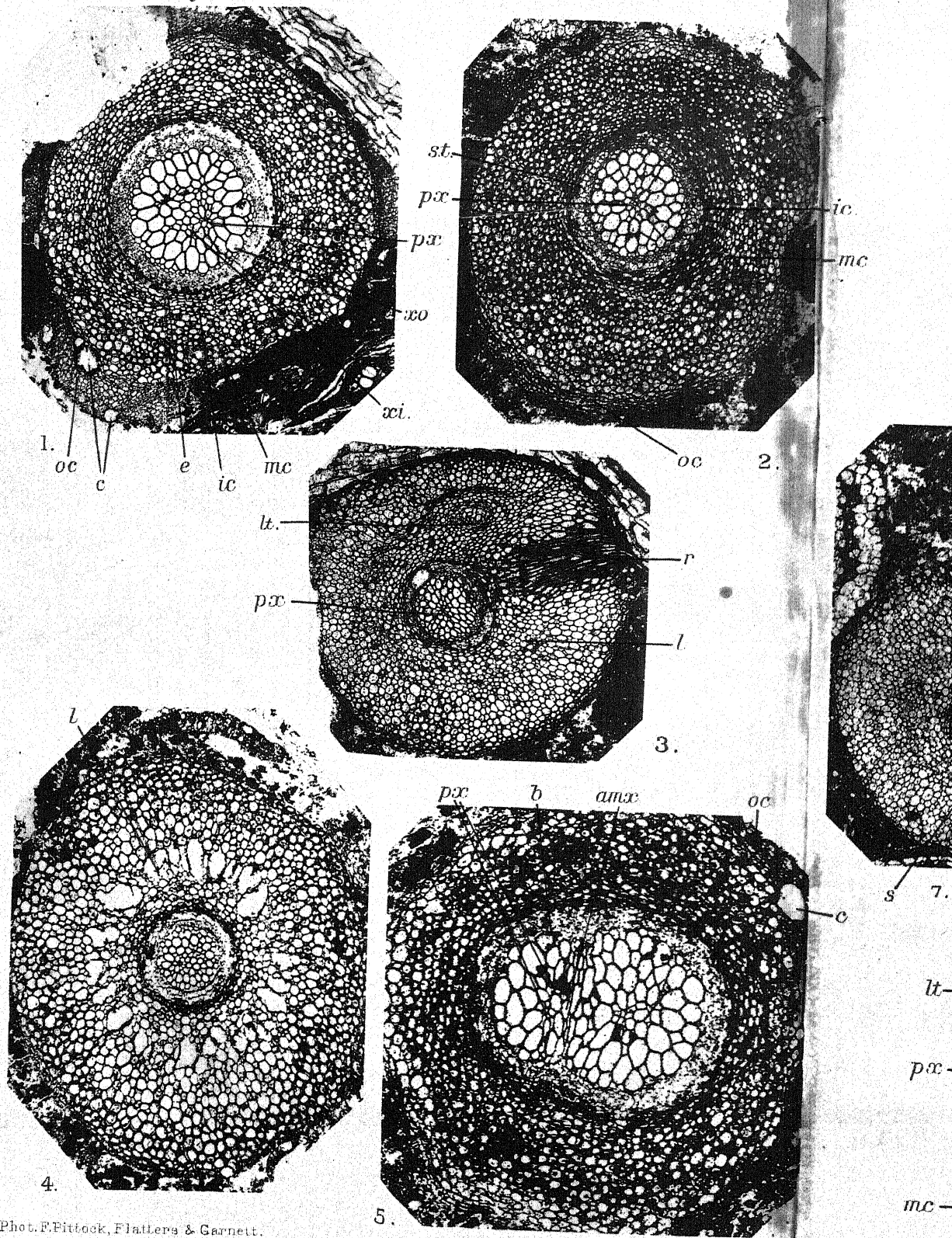
Fig. 4. A large  $\alpha$  stem, showing the origin of two roots.  $\times 40$ . (From 302.6, Dr. Gordon's collection.)



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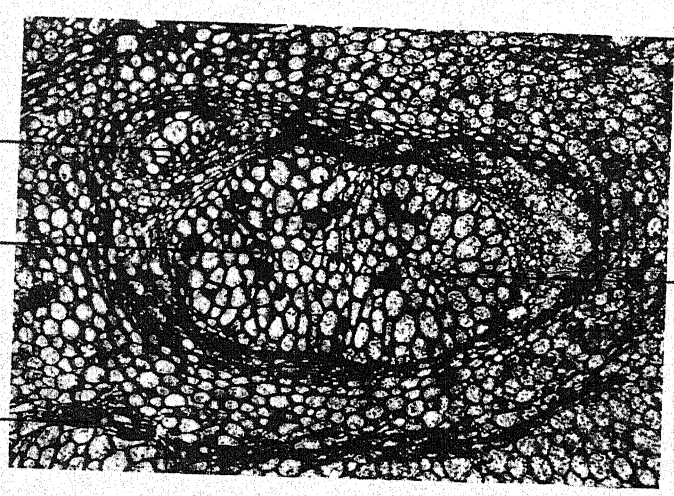
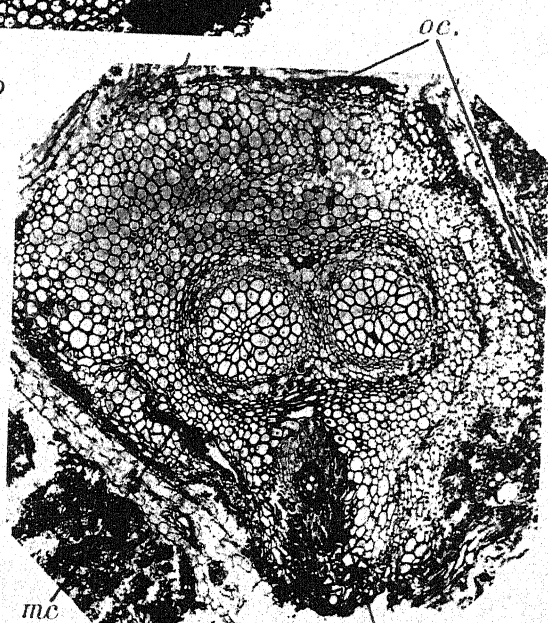
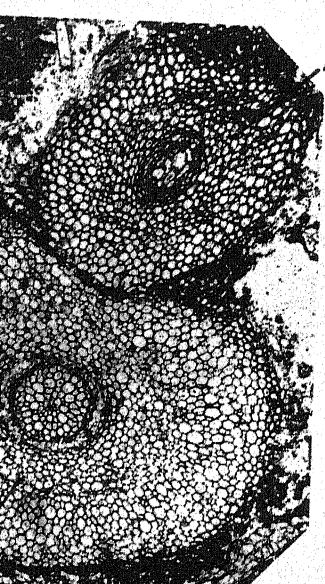
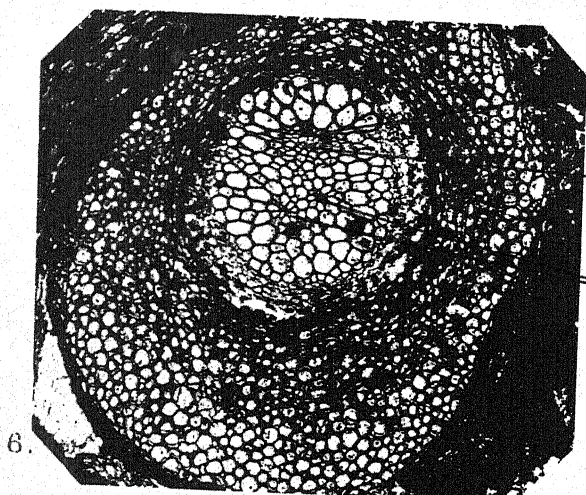
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Phot. F. Pittcock, Flatters & Garnett.

BANCROFT — RACHIOPTERIS CYLINDRICA.





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302

BOTANICAL GAZETTE

# The Origin and Meaning of Medullary (Intraxylary) Phloem in the Stems of Dicotyledons.

I. Cucurbitaceae.

BY

W. C. WORSDELL.

With ten Figures in the Text.

## INTRODUCTION.

THE object of botanical investigation, in whatever department, should be to determine, as far as possible, the interrelationship of the various facts which are accumulated, and arrange them accordingly; and not merely, as has for so long been the custom, to pile them in a chaotic heap. This is well exemplified in the case of the study of 'internal phloem' in dicotyledonous stems. By this time we have a large, chaotic heap of facts with regard to this remarkable structure, facts which sadly need co-ordination.

It has been discovered that this intraxylary phloem occurs in a large number of natural orders. In these different orders or groups it is seen to assume different forms. Very frequently it occurs as a continuous zone immediately within the xylem of the central cylinder on the extreme periphery of the pith; or this zone may be broken up into separate groups of phloem. Sometimes such a phloem-group is more or less closely attached to the inner side of each bundle of the central cylinder. At other times the medullary bast takes the form of numerous separate strands scattered throughout the pith. Again, all or some of these features may be combined in one and the same plant or natural order.

The writer's object in the present series of papers is to endeavour to demonstrate, in those cases where this is at all possible, the meaning and origin of the intraxylary phloem. It is high time that an attempt was made in this direction, if the brake on the wheels of the chariot of progress is not to hamper for ever our efforts to obtain a glimpse of the unity of Nature in this particular field of anatomical structure.

The writer considers that medullary phloem represents, probably in all cases, a *vestigial structure, the remnant of a former system of medullary vascular bundles* in which the xylem has disappeared. This has, indeed,

been hinted at by a few writers in the past, but no serious attempt ever seems to have been made to establish the theory, or to view this peculiar anatomical feature in its true light. That the surest way of determining the nature of a structure or organ is to trace its phylogenetic origin, hardly needs demonstration.

In the case of medullary phloem an attempt to do so will be made in a few natural orders where it is not hopelessly difficult. In others, however, the structure has become so stereotyped and the intermediate stages in its evolution so utterly extinct that the task is impossible.

As the first of a series of concrete illustrations of the above thesis, the order Cucurbitaceae will be taken.

#### HISTORICAL.

The following notices of work on this subject represent the more salient and relevant points brought out by the various authors:

Gérard concludes, from a study of the transition from root to stem in *Cucumis Melo* and *Cucurbita maxima*, that the internal phloem is a part of the external which becomes situated on the inner face of the bundle.

Petersen, in a general paper on the occurrence of bicollateral bundles, states that 'while the outer soft bast always forms an integral part of the bundle, this is not in the same degree the case with the inner soft bast'.

He refers to a continuous series of phenomena, ranging from a ring of bicollateral bundles to a ring of normal bundles with a whole system of medullary bundles within. He found in the creeping stem of *Alsomitra sarcophylla* that the four larger bundles of the stem almost meet in the centre, the pith being crushed, and the intraxylary phloem replaced by cambiform tissue.

Van Tieghem found in the roots of *Cucurbita* with a large pith, especially the large adventitious ones, that on the medullary side of each primary and secondary collateral bundle one or several longitudinal series of pith-cells divide actively to form a phloem-bundle.

Weiss determined that all the bundles in Cucurbitaceae are leaf-traces. The phloem-bundles scattered in the pith of the stem of *Cucumis perennis* are branches from the phloem-bundles of the leaf-traces which have passed into the outer ring. In the petioles, where the bundles are arranged in a half-moon shape, at the point where the leaf-veins branch off from the main trace, the internal unites with the outer phloem, so that the smaller bundles are no longer bicollateral.

Fischer, in a paper on the sieve-tube system of this order, traced, in *Cucurbita Pepo*, the transition from hypocotyl to root structure, and found that the medullary phloem gradually died out, ending blindly below. In the female peduncle the small collateral and the phloem-strands, which are associated with the bundles of the inner of the two rings, frequently



anastomose with the latter; after fertilization they become obliterated, and the conduction of plastic substances is thereafter carried on by the usual phloem parts.

Hérail states that the Cucurbitaceae are the only plants which have bicollateral bundles, for the development of all three parts of the bundle is identical and synchronous. He noted in *Zanonia sarcophylla* the occurrence of interfascicular cambium connecting the bundles of the outer and inner rings into a single ring.

Lamounette has an interesting thesis on the morphological origin of the internal phloem in *Cucurbita maxima*. He found that in the region between the 'heel' and the first rootlets of the young hypocotyl the external phloem had developed considerably, while the internal phloem was being separately initiated by a few divisions in the parenchyma of the pith. No communication was observed between the two. The formation of the internal is subsequent to that of the external phloem. The above applies also to *Cucumis* and *Luffa*. He concludes (not only for Cucurbitaceae but for other orders investigated) that internal phloem is an abnormal formation due to the activity of certain cells of the central conjunctive parenchyma, or is the result of the ulterior evolution of these cells; it has been acquired during evolution and then transmitted by heredity. In the cotyledon and leaf of Cucurbitaceae the internal is also of later formation than the external phloem; it does not pertain to the procambium, but has a distinct evolution from the parenchyma. He says the term 'bicollateral' should be abolished in view of the origin of the internal phloem. The Cucurbitaceae afford the best example of the acquired secondary dependence of the internal phloem on the bundle of the ring; its more primitive condition is as an independent bundle in the pith.

Scott and Brebner found in *Thladiantha dubia* that the internal phloem connects with the external in the medullary ray. In a valuable study of the course of the medullary phloem in plants generally, they found that this tissue, during the transition from stem to root, passes out and unites with the external phloem. This agrees with what has been observed in *Lagenaria* in the present paper.

Flot, like Lamounette, will give no quarter to the term 'bicollateral', in view of the fact that the internal phloem arises independently from the perimedullary zone.

Baranetzky found in the stem of *Rhynchoscarpa dissecta* that an inverted medullary collateral bundle which ran through one internode and part of another, was separated from the bundle of the ring by two or three layers of medullary parenchyma. Some only of the medullary bundles possessed xylem, and this usually died out in some region of the internode in following the bundle either upwards or downwards.

In *Bryonia dioica* and *Zehneria suavis* the same facts with regard

to medullary collateral bundles were noted. In the node the xylem of the medullary bundles unites with that of the ring.

He says: 'The phloem-bundles situated on the inner edges of the normal vascular bundles of these plants [Cucurbitaceae] are structures quite analogous to the internal vascular bundles in the stems of *Rumex* and *Rheum*. The internal phloem-bundles in Cucurbitaceae, when provided with their own wood, represent, doubtless, independent vascular bundles.' Their independence is also shown by their branching and by their passing from one normal bundle to another. He reaches this conclusion by the method of comparative histology.

He found that the differentiation of the first sieve-tubes in the internal phloem of *Bryonia alba* occurs much later than in the external phloem; but in the same plant their development may be much earlier. The development of the internal phloem-bundles of the Cucurbitaceae is just the same as that of the medullary bundles of Polygonaceae, &c.

'The appearance of internal bundles in Dicotyledons should be regarded not as an anomaly in this type, but rather as the ulterior development and perfecting thereof.' He regards it as an evolutionary development and cites its occurrence in some Gamopetalae in support of the idea.

Wallace studied the stem structure of *Actinostemma biglandulosa*, in which he found that the bundles are primarily *collateral* and remain so until after a considerable quantity of secondary tissue is formed. Two of the five inner bundles are very small, and at first possess phloem only; later on they acquire xylem, and still later become bicollateral like all the larger bundles of the two rings. Medullary phloem does not arise simultaneously in relation to all the ten bundles of the internode: the three large inner ones first acquire it, then the larger of the two inner bundles, next, those of the outer ring, and finally the remaining inner bundle. The wood of the normal bundle becomes surrounded by phloem. Medullary phloem does not accompany the leaf-traces into the leaf. The older petiole has collateral bundles.

Pitard found in *Cucurbita Pepo* tertiary phloem-strands in the rays of the stem at the edge of the wood; they are connected by branches with the internal-phloem groups of the bundles.

Faber, after tracing the development of the stem-bundles of *Cucurbita Pepo*, concluded that the internal phloem arises very early at the growing point; he found that the inner and outer phloem already existed before any vessels were formed. The sieve-tubes of the inner phloem arise from the same procambial strand as the rest of the bundle. He could discover no difference, either in the development or structure, between the outer and the inner phloem. The development of both is centripetal, i. e. towards the protoxylem. In *one* bundle only did he see two small xylem-elements formed from the cambium attached to the inner phloem. He says: 'It is

merely a matter of terminology as to whether such a bundle (the bicollateral) must be regarded as two bundles lying side by side, of which one has developed phloem only, or whether the bundle must be called bicollateral; the nature of the bundle is not thereby changed. I do not see why it should not be called bicollateral, as this better expresses the single character of the strand; the development shows that the second phloem belongs to the normal bundle.'

Col states that their masked origin and rapidity of formation has led to the Cucurbitaceous bundles being passed as bicollateral.

#### ORIGINAL OBSERVATIONS.

##### *Preliminary Remarks.*

As a result of his previous anatomical investigations in other plants, the writer has long been convinced that, in order to discover data which may throw light on the origin and meaning of an anatomical structure of doubtful interpretation occurring in the vegetative shoot, it is generally useless to study this latter from the point of view of its developmental data, whether culled from the seedling stem (either in its epi- or hypocotyledonary regions) or from the apical meristem of the adult stem. For these data are likely to throw a minimum of light, or none at all, on the nature of a doubtful structure; on the contrary, they are often very misleading.<sup>1</sup>

The conviction, on the other hand, was reached that a far more useful mine of information lay in a study of the *mature* stem, and especially of the more conservative organs of the plant, such as the *peduncle* and the various appendages of the reproductive axis, as also the *foliage-leaf*. These organs, having undergone less modification in the course of evolution of the plant as a whole, are likely to exhibit in their structure more ancestral features, and to reveal the particular character, whose morphological value it is desired to estimate, in a form nearer to that from which it originally sprang than can possibly be the case in the vegetative axis.

All these conclusions, previously arrived at, have been confirmed as a result of endeavours to throw light on the origin of the internal phloem in Cucurbitaceae, as will be seen from what will now be brought forward. In one or two cases, however, it will be noted that a study of the vegetative stem affords most of the necessary data, as in *Acanthosicyos* and *Ecballium*. Brief accounts of seedling structure are given in one or two instances, more for the sake of completeness than anything else.

<sup>1</sup> Hérail's reliance on the position and mode of ontogeny of the internal phloem is, from the morphological view-point, entirely useless.

*Lagenaria vulgaris.**Seedling.*

In the *hypocotyl* occur six bundles, having the usual bicollateral structure, surrounding a central lacuna. The transition from stem to root structure takes place below the 'heel', approximately in the region where the first lateral roots are given off. In tracing the structure downwards by means of a series of transverse sections, the internal<sup>1</sup> phloem-groups pass outwards and unite with the outer phloem-groups immediately before the transition to root-structure occurs, and therefore just before the junction of the lateral roots. As each phloem-strand passes out it revolves on its axis through 180°. The phloem-strands do not all pass out exactly at the same level. When two strands occur opposite the protoxylem of a single bundle, they pass out on opposite sides of the latter. A fairly wide pith is present even after root-structure is formed.

The fate of the internal phloem-groups is thus very different from that of those in the hypocotyl of *Cucurbita Pepo*, according to Fischer's data, and in *Cucurbita maxima*, according to Lamounette.

From such developmental facts as have just been given Gérard arrived at (what will be seen to be later) his erroneous conclusions.

There is, however, one developmental feature of some importance. Various observers, besides the present writer, have noted that the internal phloem arises *at a later period* than does the external phloem. It is commonly found that vestigial structures arise *later* in the ontogeny than is the case with other parts of the tissue-plexus. This fact would tend to indicate, therefore, that the internal phloem is a vestigial structure. As far as this goes some little clue has been gained from a study of the ontogeny. However, Faber's observations (see above) point in exactly the opposite direction.

*L. clavata.**Stem.*

The zone of sclerotic fibres, which is present in all Cucurbitaceous stems, always marks the limit of the central cylinder. This is an important character. Exactly the same is true for most, if not all, Monocotyledonous stems.

In this plant, immediately within the sclerotic ring, occur great numbers of rudimentary phloem-strands.

The central cylinder consists, as in so many Cucurbitaceae, of two circles of bundles. At the sides of the bundles of the inner ring, or, in

<sup>1</sup> The term 'internal phloem' will, in the following pages, always be applied to the phloem-strand which is attached to the ventral or inner side of the 'bicollateral' bundle of the cylinder. To all other phloem-strands occurring in the pith or the embouchement of the rays the term 'medullary phloem' will be given. These distinctive terms are for purposes of clear description.

other cases, nearly half-way between the two rings, are smaller bundles possessing a very small amount of xylem. One of these bundles, as seen in a transverse section, taken near the node, had *several xylem elements attached to the outer*<sup>1</sup> *side of its internal phloem.*

At the side of one of the bundles of the inner ring or series was seen a small isolated phloem-strand. This fact is important: it shows the occurrence of phloem-strands which represent independent bundles; for this strand is obviously homologous with a small bundle, possessing a small amount of xylem, which was described in the last paragraph. It has thus, most probably, lost its xylem and is on the way to extinction.

#### *L. leucantha.*

##### *Petiole.*

Besides the cylinder of normal bundles there are three quite small bundles, one of which, occurring in the ring of large bundles, possesses a little xylem. The remaining small strands, occurring in the pith, possess phloem only; if traced higher up, one of these latter is seen to fuse with the internal phloem of one of the bundles of the ring; the other two appear to die out above. In the highest part of the petiole, where the medullary cavity occurs, there is no sign of any of the small strands. These latter evidently represent an interior system or ring of vascular bundles.

#### *L. clavata.*

##### *Peduncle.*

In the middle typical part of the organ there is a central pith-cavity surrounded by an irregular, sinuous ring of bicollateral bundles, evidently formed by the radial congestion of two rings. A portion of one of the bundles has its internal phloem widely separated from the xylem and from the internal phloem of the major portion of the bundle; this medullary phloem-strand is thus quasi-independent. At the side of one of the ring-bundles, near the inner embouchement of the ray, is a very small normally-orientated vascular bundle. At the inner embouchement of another ray, and alongside the internal-phloem group of the adjoining ring-bundle, is a very small *inverted* vascular bundle. 'Its position and appearance suggest that it represents a fellow-strand of the internal phloem of the ring-bundle. This is supported by the fact that, *attached to the outer side of the internal phloem of two or three of the ring-bundles, are from one to a few xylem elements.* This is due to the fusion, at least in

<sup>1</sup> Wherever this term is used in the same connexion it has reference to the parts of the organ as a whole, not to those of the individual bundle.

some cases, of a small inverted bundle with the internal-phloem group. This phenomenon will be referred to hereafter in the case of another plant, and will then be commented on, as it is a fact of first importance.

In the basal region of the peduncle the ring of bundles is quite normal, and none of the small bundles occur.

### *Cucurbita Pepo.*

#### *Seedling.*

The transition to root-structure occurs just below the 'heel'. As the protoxylem of each bundle divides and turns outwards, portions of the internal-phloem strand also branch off on either side and pass out to unite with the external phloem; this is easily observed, as the albuminous cells are particularly abundant and well-defined. This 'passing out' of the internal phloem is deduced from the fact that at this level connexions between the internal and external phloem occur, which phenomenon is not present above and below this region, and that the internal phloem decreases greatly in amount at a *lower* level. There would not occur the above-mentioned connexions of the external with the internal phloem if the latter merely died out *in situ*. The connexions, consisting of groups of albuminous cells in the rays between the bundles, therefore indicate a passing out of a portion of the internal phloem. Now, it is an interesting fact that not all the internal phloem passes out. The protoxylems revolve; and a *continuous cylinder* of wood is formed before the latter process is complete; so that scattered elements of internal phloem are left behind and enclosed in the wide pith, and eventually die out below. The conclusions of Lamounette and Fischer are thus only very partially correct in regard to this genus. The former relied far too much on the misleading data of the ontogeny.

#### *Stem.*

In the subaerial portion, some distance beyond the yellow-coloured underground part, the cambium of the internal phloem has, in most of the bundles of the ring, developed a large amount of xylem which consists mainly of parenchyma, with vessels and fibres in some of the bundles. The phloem of these bundles is connected with that of the ring-bundles by commissural strands; the woody part of the xylem appears to die out at a lower level, to reappear again in the yellow underground portion.

In another plant, at the lowest node, a few inches above the base of the stem, where the yellow portion of the latter begins, the internal-phloem groups send off branches abundantly both to the external phloem

and to each other. The wide rays are filled with separate phloem-strands. The internal-phloem groups also give off smaller branches, pursuing a very irregular twisting course, into the pith, where small phloem-groups are seen to occur; some of these latter possess, at least during part of their course, xylem.

This structure of the node is not a haphazard one. The node is the most conservative portion of the vegetative stem. In the absence of any convincing proof of the presence of a physiological cause to account for the supernumerary medullary phloem-strands and bundles, their presence may be regarded as an ancestral trait, probably representing the vestiges of a former medullary bundle-system. The fact that they belong to the same system of strands as the ordinary internal-phloem strands of the ring-bundles, shown by their fusions therewith, is an indication that the ordinary internal-phloem strands really represent independent bundles which have, in the majority of cases, lost their xylem through degeneration.

#### *Peduncle of Male Flower.*

In the upper part one or two of the bundles of the ring have a few primary xylem elements on the pith-side of the internal phloem, which, lower down, along with a little phloem, pass off as a small medullary bundle, whose xylem, at a still lower level, dies out. In other words, a phloem-strand of the pith, if traced *upwards*, becomes a vascular bundle, which, at a still higher level, fuses with the internal phloem of one of the ring-bundles. This phloem-strand was not traced to its conclusion in the lower part of the peduncle; it probably ended blindly in the pith.

#### *Peduncle of Female Flower.*

In a young peduncle examined there were seven phloem-strands situated in the pith near the bundles of the cylinder; they were about the same size as the internal-phloem strands of the latter, but quite circular in shape. Some have a smaller phloem-strand lying near them; these smaller strands also occur in the rays and pericycle, and are very numerous; farther down they either fuse with the larger strands (internal-phloem groups or medullary ones) or else die out *in situ*. The larger medullary strands were not followed to their ending. Some of these latter have a vessel or two attached to them, chiefly on the *outer* side.

In a mature peduncle bearing a fruit it was observed that in the upper region below the fruit occurred great numbers of small variously-orientated *vascular bundles in the pith*, nearer the margin than the centre of the latter, as also along the inner sides of the ring-bundles at the embouche-

ment of the rays. Three of these bundles were traced downwards; from one of them, whose xylem was directed *outwards*, three or four minute phloem-strands became separated off, which eventually fused with an internal-phloem group. The bundle itself fused with one of two bundles lying opposite the adjoining ring-bundle, the product of fusion farther down fusing with this ring-bundle, i. e. with its internal phloem; the remaining one of the two bundles just mentioned dwindled greatly

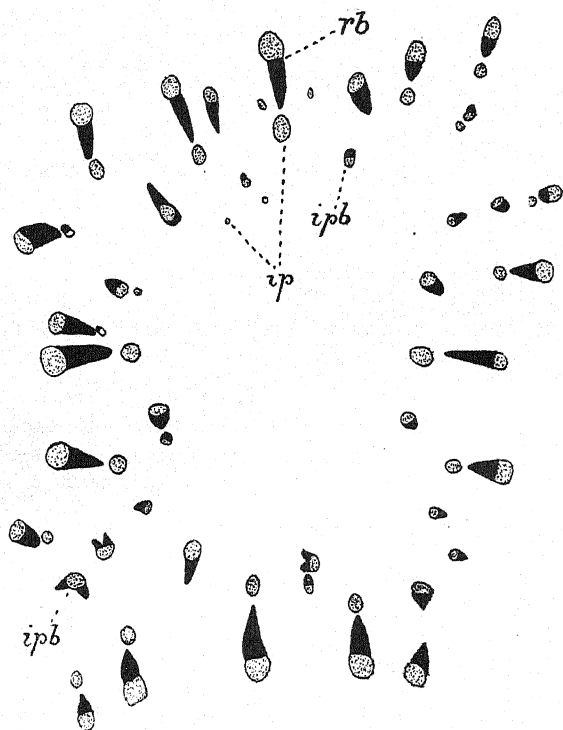


FIG. 1. *Cucurbita Pepo*. The vascular tissue of the fruiting peduncle, in transverse section, showing scattered and independent internal-phloem strands and bundles; the latter variously orientated, and one or two with amphivasal structure. *rb*, vascular bundle of the normal ring; *ip*, internal phloem; *ipb*, medullary bundle or bundle of abnormal ring, homologous with 'internal phloem'.  $\times 11$ .

in size, and finally appeared to die out *so close* to the large internal-phloem group that it practically amounted to a fusion therewith.

Another small inverted medullary bundle, on being traced downwards, was seen to fuse with the internal-phloem group opposite to it; after the fusion the xylem of the former occupied a lateral position in the resulting large phloem-group; farther down it appeared embedded in the middle, remaining so as far as it was traced, so that the phenomenon occurred of an internal-phloem group with central xylem.

In another fruit-peduncle there were one or two rings of large bundles



composing the cylinder, with here and there much smaller bundles in the pith closely adjoining them or on their sides; these small bundles are inverted collateral or amphivasal in structure. The internal phloem of the large bundles of the ring has the form of a detached, large, rounded strand encircled by a cambium which, in nearly all cases, has formed some tissue which has the characters of the xylem parenchyma of the main bundle; it is usually most greatly developed on the *inner* (pith) side; in only one case was a *small group of vessels* seen attached to the inner side of the internal phloem; but the soft-walled tissue formed by the cambium may be regarded as xylem. Not only are there distinct evidences of the scattered arrangement of the bundles of the cylinder, but there is also a distinct tendency in these towards amphivasal structure, for the bundle whose internal-phloem group possessed vessels was concentric, the phloem being completely encircled by the xylem; all the other bundles are very V-shaped.

The above-described fusion of a small medullary vascular bundle with an internal-phloem group leads to the conclusion that the latter represents an independent vascular bundle which has lost its xylem, for the medullary bundle becomes one with it, and the internal-phloem group becomes, at a lower level, a vascular bundle.

No case is known in any plant of a vascular bundle fusing with the *outer* phloem of a bundle of the cylinder. Hence the internal phloem is not the equivalent of the outer phloem, in the sense of forming a constituent part of the bundle of the ring, but must be regarded as an independent bundle of the pith. This view is again strongly supported by the facts recorded in the second fruit-peduncle: the independent character of the rounded internal-phloem group, with its parenchymatous and, in some cases, woody xylem. It clearly represents an incompletely formed amphivasal vascular bundle, and is probably a vestigial structure. The bundles of the cylinder are also clearly more or less perfect or imperfect amphivasal bundles. The whole thus constitutes, in the writer's opinion, the *vestige of a scattered system of bundles* composed of several series or irregular rings; the internal-phloem groups attached to each ring of bundles would represent always, on this view, a distinct series or ring of bundles (cf. Fig. 9).

#### C. foetidissima (Cucumis perennis).

##### Stem.

In the extreme basal region is a single bundle-ring of quite cylindrical contour and composed of about twelve bundles of various sizes and rather closely approximated, the large internal-phloem masses occupying almost the entire pith. At a higher level the number of bundles is greatly increased and the ring is no longer of cylindrical contour, but exceedingly sinuous, consisting of five arms. In the outer or pericyclic zone of the

cylinder occur great numbers of small phloem-strands; of these some, viz. the most rudimentary, occurring immediately within the sclerotic ring, are those met with in this region in most Cucurbitaceae; but others, representing an extension of this system, are larger, of varying size, irregularly grouped, and occurring chiefly between the protrusions of the bundle-ring. Those, however, which approximate to the ends of the latter, and thus tend to form part of the bundle-ring, *possess some xylem*. It is thus evident that there are transitions between the tiny phloem-strands of the extreme outer edge of the cylinder and the vascular bundles of the ring. It was not ascertained whether the tiny outer vascular bundles unite with the bundles composing the ring-protrusions or whether they die out at a higher level;

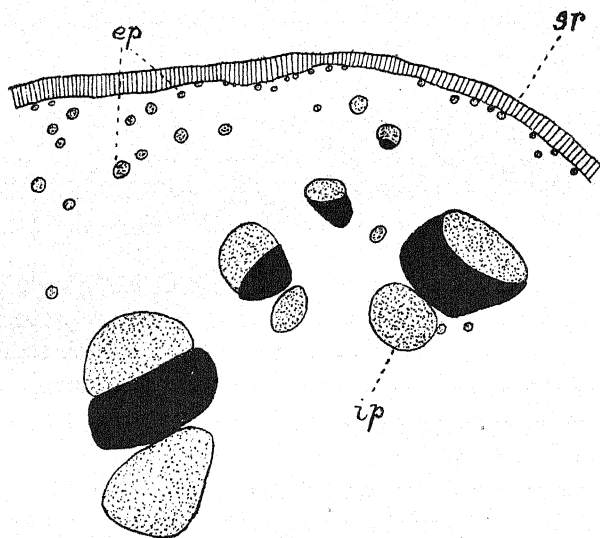


FIG. 2. *Cucurbita foetidissima*. Transverse section of a small portion of the central cylinder of the stem, showing a transition between the bundles of the ring and the small phloem-strands (*ep*) of the extreme periphery of the cylinder; these latter representing vestiges of former vascular bundles. *ip*, 'internal phloem'; *sr*, sclerotic zone.  $\times 27$ .

this is immaterial; it is certain, however, that the tiny phloem-strands do die out at a higher level.

We may conclude that the tiny phloem-strands and bundles described represent the vestigial remains of one or more outermost series or rings of bundles. In other words, there are in this stem clear traces of an ancestral scattered bundle-system, such as occurs in Monocotyledons. The peculiar sinuous contour of the bundle-ring, so characteristic of the Cucurbitaceae, can also be explained. It represents an attempt to condense all the rings or series into a single ring, the contour of which becomes more and more cylindrical and even and the individual bundles (or some of them) larger as the base of the stem is approached. The sclerotic ring, situated,

as a rule, near the periphery of the stem, just as in Monocotyledons, limits as is the case in this class, the central cylinder.

In this species we thus discover in the stem the existence of vestigial remains of *outer* series of bundles pertaining to the original scattered system; in the last species we found the vestiges of an *inner* series of bundles belonging to the same scattered system.

**Cucumis sativus.**

*Stem.*

As far down as a few millimetres or so below the point at which the green portion of the stem terminates the structure offers nothing of particular interest. But at about that level, where the stem has become very deeply lobed and fluted into six columns, each of which is traversed by a bundle, the majority of these bundles has each, *in place of the ordinary internal-phloem strand, an inverted vascular bundle*,<sup>1</sup> often more than half its own size, a large amount of xylem being present, including vessels and fibres.

In another stem the external phloem is connected with the internal by 'commissural strands' which also often have a cambium and a few xylem elements formed at the flanks of the xylem of the main bundle; this latter is often quite surrounded by phloem.

It is thus in the *lower* part of the stem only that the internal-phloem strand is represented by a vascular bundle.

**C. echinophorus.**

*Petiole.*

In the typical region, where the groove occurs on the upper side, is a complete circle of bundles; but those on the median upper side opposite the groove are rudimentary, having one or two or no xylem elements and a small amount of phloem; two, at any rate, are represented by small phloem-strands only. One of the bundles near the end of what (when the ventral bundles have died out lower down) will become the arc has several xylem elements developed on the outer side of the internal phloem, forming a quite distinct and individualized inverted bundle.

**Cucumis Melo.**

*Peduncle of Fruit.*

The double series of bundles is practically constituted as a single ring.

A few of the bundles have *two* internal-phloem strands, one behind the other in the same radial line; in one case the extra (innermost) strand

<sup>1</sup> Solereder makes no mention of this.

appeared to be double. These facts also strongly support the view that the internal phloem represents an independent bundle.

Two or three of the internal-phloem strands possess woody elements (vessels and fibres) on their *outer* side, a fact which of course rounds off the evidence, already partly supplied by the above-mentioned facts, that the internal phloem in this genus represents an independent bundle which has, for the most part, lost its xylem.

### *Citrullus vulgaris.*

#### *Seedling.*

In the transitional region between the hypocotyl and the root the internal-phloem strands, of which each bundle may have two or three, pass out along the side of the bundle and unite with the external phloem, leaving the bundles completely free from internal phloem before the protoxylem begins to rotate.

This phenomenon, which was also noted in *Lagenaria*, appears to prove that the *internal-phloem strand is not a constituent part of the bundle*, as it moves quite independently of the latter, and at a different time, behaving, in fact, exactly like an independent medullary bundle.

### *C. ecirrhosus.*

#### *Stem.*

This plant was collected by the writer in the Namib desert of Damara-land in 1910. The stem is prostrate and trailing, with a structure like that of a root, for there is hardly any pith. There is a large amount of secondary wood, with wide-lumined vessels.

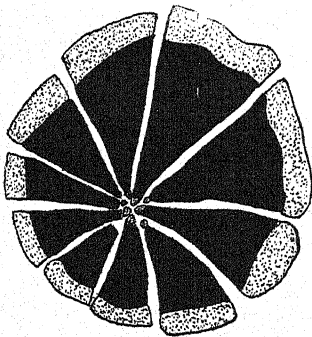


FIG. 3. *Citrullus ecirrhosus*. Transverse section of the ring-bundles and internal-phloem strands of the stem.  $\times 11$ .

The usual internal phloem is present.

The rays are wide.

The limit of the cylinder is indicated by isolated groups of fibres.

#### *Peduncle of Fruit.*

In the swollen part of this organ immediately below the attachment of the fruit is a ring of bundles" possessing not nearly so much secondary wood as in the case of the stem-bundles, and enclosing a somewhat wider pith. The *internal-phloem strands of the stem are here*

represented by vascular bundles which clearly belong to the amphivasal type, but, in some cases, are reduced therefrom, having xylem on their *outer* side

only; one or two, however, have xylem completely, or in the case of others incompletely, surrounding the phloem. The cambium entirely surrounds *all* the bundles. The outer portion of the xylem of these bundles abuts very closely, only separated by one to three parenchyma elements, on the protoxylem of the ring-bundle. The xylem of the amphivasal bundle consists for the most part of short fibres with rudimentary bordered pits in their walls, quite similar to those of the wood of the ring-bundle. But the elements nearest the protoxylem of the latter are rather shorter, with rather thinner lignified walls, covered with very numerous simple pits, and with very slightly oblique end-walls. All the internal bundles show a tendency to doubling, their xylem consisting of two arc-shaped strands more or less united in the tangential plane.

Here and there in the cylinder is a curious group of bundles: on one or both sides of one or more of its large bundles, a much smaller bundle of the cylinder occupies an oblique position in the angle between the large bundle of the cylinder and the internal (medullary) bundle; the latter is also somewhat obliquely placed. The explanation probably is that an attempt is here being made to merge the bundle of the cylinder and the internal (medullary) bundle into a more closely compacted and better organized whole, viz. into a large concentric (amphiphloic) bundle.<sup>1</sup> From the *morphological* point of view the structure indicates the ontogenetic origin of the internal-phloem bundles from the ring-bundles, for the obliquely situated strand represents one of the former which is imperfectly separated off from one of the latter. The arc-shape of the phloem of the ring-bundle causes the oblique position of the strand above mentioned; and we can find in these facts an explanation of the *inverted* orientation of the internal-phloem bundles, for if we imagine the oblique strand passing further towards the pith, it would, as we know from the analogy of similar cases, revolve further on its axis so as to eventually assume an inverted position. This is, indeed, exactly what happens when the internal-phloem strands (which represent these bundles in the hypocotyl) pass inwards from the phloem of the ring-bundle; the phloem-strand (imperfect bundle) revolves on its axis and on reaching the pith assumes an inverted position.

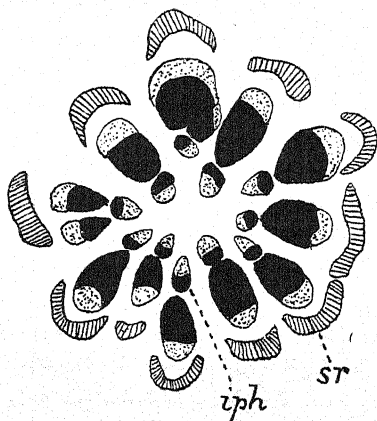


FIG. 4. *Citrullus ecirrhosus*. Transverse section of central cylinder of fruiting peduncle, showing the independent internal-phloem bundles. *iph*, 'internal phloem'; *sr*, sclerotic zone.  $\times 11$ .

<sup>1</sup> Cf. case of *Acanthosicyos* and the 'stele' of Primulaceae.

As the basal portion of the peduncle is approached the xylem of the internal amphivasal bundles gradually becomes extinct. The inner xylem, where it is present, disappears first, to be followed eventually by the outer xylem, until, in the narrow basal part, the structure is precisely that of the vegetative stem, with internal phloem only.

At the *node*, representing the point of origin of the peduncle, some of the medullary bundles have well-developed outer xylem.

The occurrence of complete vascular bundles, with inverse orientation, in the place of the internal-phloem strands, in the upper part of the fruiting peduncle is probably an adaptation to meet the structural requirements arising from the attachment of a large fruit. As the xylem of the internal bundle consists in its major portion of fibres, and exhibits very few vessels, the function is doubtless mainly a mechanical one, viz. to meet and resist tension strains, which would be severest in that part of the peduncle.

Now, if the internal phloem of the stem and lower part of the peduncle represents, on the ordinary academic view, an indissoluble constituent of the bundle of the cylinder, of no particular morphological value, the intercalation of xylem between this internal phloem and the xylem of the ring-bundle would appear strange and anomalous and no meaning could be attached to it, unless the internal phloem is to be regarded as the last vestige of a phloem which entirely surrounded the xylem in an original concentric bundle. There is, however, no evidence that the bundles of the axial organs of Cucurbitaceae were originally amphiphloic in structure. If, on the other hand, the internal phloem represents the vestige of an original medullary amphivasal bundle, then the reappearance of its xylem for the purposes above described is easily understood, for the new mechanical elements are laid down in the place, so to speak, of least resistance, i.e. where they formerly existed in the axis of the ancestor, the reversion to the primitive condition being easily invoked by the stimulus of the tension-strain.

As has been mentioned above, the tendency to form large concentric amphiphloic bundles does occur, but this is not to be regarded as having a reversionary significance, for it is purely adaptational.

### **Ecballium Elaterium.**

#### *Stem.*

In the *lower* part the internal phloem is replaced by large complete bundles separated some little distance from the protoxylem of the cylinder by ground tissue; its cambium has formed a very large amount of parenchymatous tissue, mostly pertaining to the xylem, amongst which in *every* bundle are from one to several vessels or fibres situated on the *outer* side of the phloem-strand (cf. Fig. 9).

The peduncle has nothing of interest in its structure.

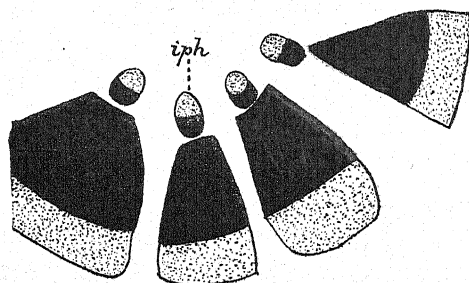


FIG. 5. *Ecballium Elaterium*. Transverse section of stem-base showing the independent 'internal-phloem' bundles. *iph*, 'internal phloem'.  $\times 11$ .

### *Trichosanthes anguina*.

#### *Stem.*

Two of the bundles of the inner of the two rings or series have no internal phloem at all, nor do they possess protoxylem, so that they may represent secondary structures.

#### *Peduncle of Fruit.*

In the inner fleshy rind of the fruit are great numbers of small variously-orientated and constructed bundles, many of which are devoid of xylem. At a lower level, i. e. in the upper part of the peduncle, the vascular system consists of two rings or series of normal bicollateral bundles, and within these, in the pith, are a few bundles representing those above mentioned, which have descended from the inner rind of the fruit. One of these medullary bundles consists of a rounded phloem-strand with xylem elements situated at intervals round the greater part of its periphery; another had a little xylem on its *outer* side, apparently formed by a cambium; others consisted of phloem only. At a lower level in the peduncle all these medullary bundles or phloem-groups unite with the internal-phloem strands of the bundles of the inner ring; at a still lower level one of these internal-phloem strands apparently had several xylem elements in its midst, due to its fusion with a medullary bundle.

If we regard the structure as traced upwards from the base, we see the internal-phloem strands of the inner ring of bundles branching and giving rise to independent phloem-strands or vascular bundles, as the case may be, which constitute a distinct medullary system; the xylem of the vascular bundles which arise in this way is apparently differentiated either, as in the above instance, before the bundle leaves the internal phloem-strand or at a later period.

The above-described structure of the peduncle is an interesting one, for,

inasmuch as the medullary bundles are shown as arising from the internal phloem-strands, it follows that *these latter must be regarded as forming part and parcel of the same system of medullary bundles.*

#### *Acanthosicyos horridus.*<sup>1</sup>

The material of this plant was collected by the writer on a sand-mound at a Hottentot village about two miles inland from Walfisch Bay in South-west Africa.

#### *Aerial Stem.*

The rigid branched stems are devoid of leaves, the place of each leaf being occupied by a pair of woody cylindric stipules in the form of spines.

The cortex is very narrow. The sclerotic zone limiting the central cylinder externally has a very sinuous outline, forming a number of deep intrusions on the flanks of and around the points of which the periderm occurs. These sclerotic intrusions correspond in number to, and are opposite, the main bundles of the cylinder. In the bays between the sclerotic intrusions occur the very small bundles, one, as a rule, in each bay, of the outer ring or series of the cylinder. Each has a small internal phloem-strand which is very loosely, i.e. far from intimately, connected with the xylem. In one bay there were three small bundles, only one of which, viz. the middle one, possessed internal phloem. In the outer (cortical) bay formed by each sclerotic intrusion occurs the green assimilating tissue of the stem.

The inner (main) series of bundles of the cylinder, about sixteen or seventeen in number, are very well-developed, and each has a large, very rounded internal-phloem strand. Of these latter one to three possess xylem, consisting of vessels and fibres and a large quantity of parenchyma, on the *outer* side between the phloem and the protoxylem of the bundle.

There is a fairly wide pith.

#### *Subterranean Stem.*

This is very thick and woody.

The same structure is found as in the aerial stem, but there is relatively less xylem attached to the large internal phloem-strands, a fact which is probably correlated with the very large amount of wood developed in the bundles of the ring.

#### *Peduncle of Fruit.*

The fruits had at this time (April, 1910) attained a fair size (about that of a croquet-ball), but were not yet ripe.

The structure of the peduncle is, in essentials, the same as that of the aerial stem. All the internal-phloem strands have, on their outer side,

<sup>1</sup> Marloth has written an interesting account of the habit and structure of this plant.



a large amount of xylem, most of which is parenchymatous, but with a small group of fibres developed towards the outer side.

On the other hand, the xylem on the outer side of the internal phloem of the *outer* series of bundles is, in almost every bundle, much more greatly developed both as regards parenchymatous and woody elements, sometimes equalling that of the bundle itself; both the external and internal phloem

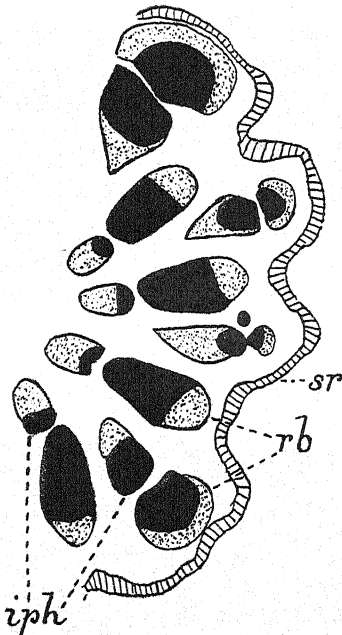


FIG. 6. *Acanthosicyos horridus*. Transverse section of peduncle, showing two rings or series of bundles, each with its internal-phloem bundles (*iph*). *rb*, vascular bundle of the normal ring; *sr*, sclerotic zone.  $\times 11$ .

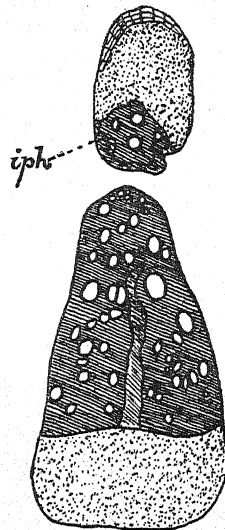


FIG. 7. *Acanthosicyos horridus*. Transverse section of a single bundle of the ring with its internal-phloem bundle (*iph*); the essential amphivasal structure of the latter is shown by the cambium situated on its medullary side.  $\times 30$ .

are very arc-shaped with the concavities, filled with the xylem, opposed to each other; the internal phloem is also much radially extended. The whole resembles a large concentric (amphiphloic) bundle incompletely built up owing to the presence of two wide rays, one on either side, representing the ground-tissue areas separating the two large vascular bundles.

#### *Stipule.*

A cylindric organ resembling a small twig in its structure.

The outer series of bundles, situated in the bays between the sclerotic intrusions, is much better developed than the inner series. The latter are more or less rudimentary, with an almost complete absence of internal

phloem. The internal phloem of the exterior bundles is well developed and separated from the protoxylem of the bundle by two or three ground-tissue elements; this fact, and the further one that most of the internal-phloem strands have xylem elements attached to their periphery at various points, shows clearly that the internal phloem of these exterior bundles represents an independent bundle, having no morphological connexion with the bundle opposite which it occurs. These internal-phloem groups of the outer bundle-series doubtless represent the vestige of a former bundle-series occurring between the two at present alone existing.

The structure of *Acanthosicyos* thus affords plenty of evidence in support of the writer's theory, and nothing which in any way contradicts it.

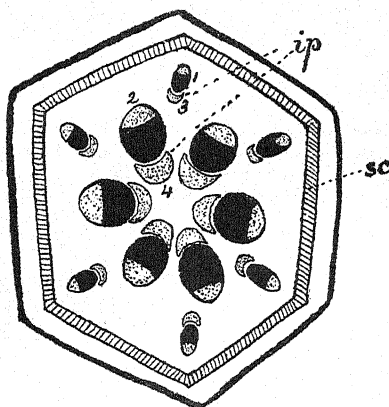


FIG. 8. Transverse section of normal stem of typical Cucurbitaceae, showing two series of bundles (1 and 2), each bundle with its strand of internal phloem (*ip*). *sc* = sclerotic ring. (Diagrammatic.)

#### SUMMARY AND CONCLUSIONS.

The following are the main results of this investigation :

1. The more conservative parts of the axial configuration, viz. the *peduncle*, and the *node* of the vegetative stem, are those in which ancestral traits in the structure are most likely to have been retained.
2. What are considered to be ancestral features have been found in these regions; but also, in some cases, in the internodal region of the vegetative stem.
3. Questions and data relating to the ontogeny, such as the development of the internal phloem (or bundle) from the same desmogen strand as the bundle of the ring; or the primary or secondary (cambial) mode of development of parts of the internal-phloem bundle, are of no value for throwing light on the origin of the internal phloem (or bundle).
4. In the vegetative stem of certain members of the order, and, as a rule, in its *lower* part only, the *internal phloem exists in the form*

of vascular bundles of which the xylem exists entirely, or for the most part, on the *outer* side.

5. Distinct vestiges of a medullary system of bundles, having the form sometimes of vascular bundles, sometimes of phloem-strands, have been found in the *peduncle* and the *node* of certain genera.

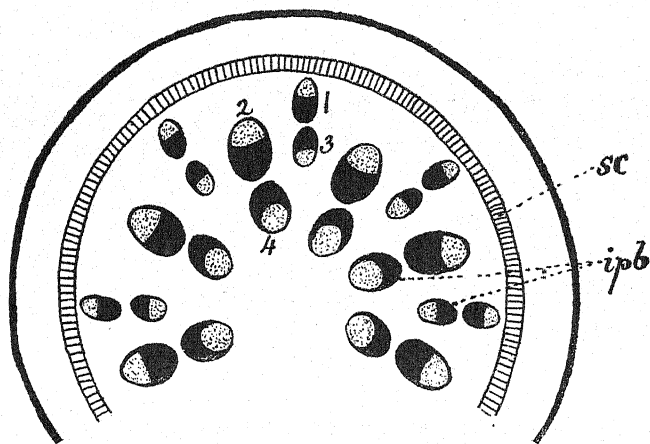


FIG. 9. Portion of transverse section of more primitive type of axis (e.g. peduncle of *Ecballium*), showing the inversely-orientated collateral bundles from which the internal-phloem strands have been derived (*ipb*); a scattered system of bundles in four series. *sc.* = sclerotic ring. (Diagrammatic.)

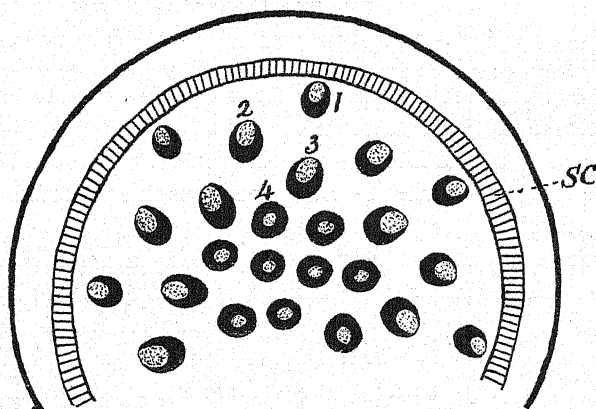


FIG. 10. Portion of transverse section of the theoretical ancestral type of stem from which that of Cucurbitaceae is presumably derived. The series of internal-phloem bundles (numbered as in Fig. 9) are here in their proper place. The innermost series are completely amphivasal. *sc.* = sclerotic ring. (Diagrammatic.)

6. The bundles or strands composing this system, on being traced upwards or downwards, either arise *de novo* or fuse with the internal-phloem strands; in any case, the vast majority unite with the latter during some part of their course.

7. The fact that the *xylem* of the medullary bundle unites with or becomes attached to the internal-phloem strand, and not with the xylem of the corresponding bundle of the cylinder, proves that the internal-phloem strand represents a distinct vascular bundle of the medullary system which has lost its xylem. No case is known in any plant of a *bundle* fusing with the *phloem only* of a collateral bundle.

8. In some cases, as in *Citrullus ecirrhosus*, where the internal phloem of the stem is normal, that of the fruiting peduncle has xylem attached to its outer side.

9. In this case a physiological need has been the stimulus evoking what is regarded as a reversion to an ancestral structure.

10. In the peduncle of *Acanthosicyos* the xylem of the internal phloem of the *outer* series of bundles is very greatly developed, much surpassing that of the inner series. In the twig-like stipule of this plant the internal phloem of the outer bundles is clearly seen to be a distinct and individualized bundle, for the reasons above given.

11. The imperfect or rudimentary structure of the intrapericyclic and medullary phloem-strands and of the medullary bundles shows them all to be ancestral vestiges and not structures in the course of evolution; for such *imperfectly functional* structures would not have been evolved and preserved. Hence the view that the cylinder has been derived from a former scattered system of bundles is correct.

12. The internal-phloem strands, although, as the writer considers, vestigial, are as well developed and functional as the phloem of the ring-bundles, owing to their having been retained as a useful and necessary adjunct to the conducting system. This has led a few authors to suppose that they represent a new product of evolution.

13. The fact that the internal phloem arises in the course of ontogeny, at a *later* period (as a general rule) than does the phloem of the ring-bundles, is in favour of its being vestigial.

14. In the only cases investigated for this purpose, viz. *Lagenaria*, *Citrullus*, and *Cucurbita*, it was found that during the ontogeny of the stem, viz. in the hypocotyl, the *internal-phloem strand arises* (as a whole in the case of the first two genera, in part in the case of *Cucurbita*) *from the phloem of the ring-bundle*. This, of course, is the natural and only mode of origin in view of the fact that the internal phloem is still fully functional and well developed.

15. The internal-phloem strand (at any rate in the cases of *Lagenaria* and *Citrullus*), as it passes inwards from the phloem of the ring-bundle, revolves on its axis through an angle of  $180^{\circ}$ .

16. This affords the *ontogenetic* origin of the inversely-orientated internal-phloem bundle.

17. The *morphological* origin of this internal-phloem bundle is from

an *amphivasal* bundle, for this latter is the typical and primitive condition of medullary bundles wherever they may occur (Fig. 10).

18. Owing to the fact that some of the outermost amphivasal medullary bundles have become approximated to the bundles of the ring to form would-be constituents of this latter, only that portion of the xylem of the original amphivasal bundle has been retained which is the most mechanically serviceable for the ring as a whole, viz. the *outer* portion, or that which is approximated to the xylem of the ring-bundle.

The inversely-orientated internal-phloem bundle is thus explained.

19. In the vast majority of cases the xylem which occurs on the outer side, or other parts of the periphery, of the internal phloem is *secondary* in origin, i. e. is derived from a cambium. In a few cases, as in that of the outer series of bundles in the stipule and stem of *Acanthosicyos*, it appears to be, at least part of it, primary. The mode of origin of the xylem, whether primary or secondary, is, however, a matter of purely ontogenetic interest; it cannot affect the morphological question as to the origin of the internal phloem, and is, in this connexion, of no importance.

20. This fact of the existence of vascular bundles replacing the internal phloem in the stem and peduncle proves that the 'bicollateral' bundle has no existence in the morphological sense, but is a purely descriptive term.

21. The 'bicollateral' bundle of the Cucurbitaceae is a compound structure consisting of the more or less intimate association or attachment of two distinct vascular bundles, of which the innermost has lost its xylem.

22. The collateral bundles composing the two rings or series of the cylinder in this order also, in the writer's opinion, represent reduced amphivasal bundles. In some plants they are very V-shaped, with the phloem situated between the arms of the xylem. In this feature and in that of the large size of the vessels the bundle is sometimes an exact replica of that of some Monocotyledons.

23. In most Cucurbitaceous stems there may be observed in the region outside the zone of the two main series of cylinder bundles, but within the sclerotic ring, a few extremely rudimentary phloem-strands. In *Cucurbita foetidissima* evident transitions between these and the bundles of the cylinder exist in the form of intermediately-situated vascular bundles.

24. This last fact shows that the external rudimentary strands represent the vestiges of a former outermost series of bundles of the cylinder.

25. The existence of the rudimentary phloem-strands just mentioned representing reduced vascular bundles helps, in some degree, to render plausible the view that the *internal*-phloem strands also represent reduced vascular bundles.

26. The sclerotic ring, broken up in some cases into isolated strands, marks the limit, as in Monocotyledons, of the central cylinder.

27. The conclusion is reached that the vascular system of Cucurbitaceae represents *the vestige of a former ancestral scattered system of bundles* such as obtains in Monocotyledons, of which only two series or rings remain in perfect condition, the rest appearing in the form of rudimentary external phloem-strands (rarely bundles as well), 'internal-phloem' strands, and medullary bundles or phloem-strands (Figs. 8, 9, 10).

28. The sinuous contour of the bundle-ring in most Cucurbitaceae is the expression of the partial congestion of two bundle-series into one—an intermediate condition between the cylindric and the scattered system.

29. Such a structure as the internal-phloem strand must be investigated both from the morphological and physiological standpoints. Its function, ontogeny, and phylogeny must all equally receive consideration.

The writer is indebted to the authorities of the Royal Gardens, Kew, for much of the material used in this study.

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# On the Ribbing of the Seeds of *Ginkgo*.

BY

MISS M. F. A. AFFOURTIT

AND

MISS H. C. C. LA RIVIÈRE.

With a Figure in the Text.

SOME months ago we received a number of seeds of *Ginkgo biloba*, gathered from a tree growing in a garden at Slikkerveer, in the neighbourhood of Rotterdam.

When the thick, fleshy portion of the seeds had been removed, they showed their stony coats; it was remarkable that these were then of very different shapes, even more different than there was reason to anticipate from what is mentioned in the literature. Generally, the seeds seem to be considered as two-sided, but it appears that already A. Braun (1) noticed a difference in the shape of the stones; as he says (p. 738): 'Bei *Ginkgo* sind die Samen normal zweikantig, zuweilen sehr regelmässig dreikantig.'

E. Strasburger states (2, p. 15): 'Das Endocarp erscheint, entsprechend der Gestalt des inneren Raumes, scharf zweikantig, seltener dreikantig.'

In G. de Saporta and A. F. Marion we found (3, p. 139): 'Sous un mésotesta charnu, elle renferme un endotesta osseux en forme de coque bi- ou tricarénée.' They also give drawings of a two- and of a three-angled seed (Fig. 69, E, F).

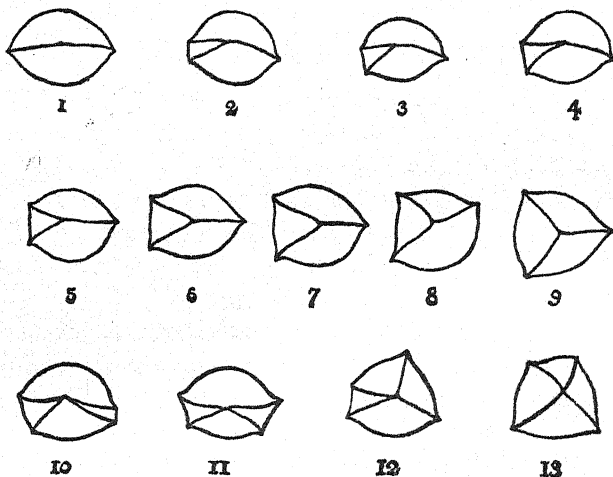
A. W. Eichler (4, p. 109) speaks only of a two-angled form of the stony coat, and figures it (Fig. 68, e, f).

In the more recent literature the three-angled forms are referred to, as for instance in the following publications: A. C. Seward and J. Gowan (5, p. 124), Carothers (6, p. 126), and A. Sprecher (7, p. 122). The latter gives also a drawing (Fig. 124) of a three-sided seed, and writes: 'La plupart des noyaux scléreux ont deux côtés, mais on en trouve assez fréquemment qui en ont trois.'

The relatively large number of seeds we had at our disposal (viz. 117), all derived from a single tree, did not however show such regular forms as might be supposed from the published descriptions, so that we thought them worth placing on record. For not only did they show

many gradations between those two regular kinds, i. e. the two- and three-ribbed types, but there were also other forms, still undescribed, as far as we are aware.

Whilst nearly half the number were two-angled, even these were not quite regular in form; three kinds of them could be distinguished, though the differences between them were rather slight. In most cases the sides were equally curved, as in the drawings of Eichler and of Saporta and Marion, and as in our own Fig. 1, but in some other cases one of them was evidently smaller and much flatter than the other. In these cases the angle which the two ribs made at the summit and base was always  $180^\circ$ , so that the two ribs formed one straight line, as in Fig. 1; but in a third form



Thirteen kinds of seeds of *Ginkgo*, from which the fleshy coat had been removed; all seen from the top; natural size. Fig. 1. Two-angled seed. Figs. 2-9. Three-angled seeds. Figs. 10-13. Four-angled seeds.

this angle was smaller, varying for instance in three cases between  $135^\circ$  and  $160^\circ$ .

There was also a large number of three-angled seeds, even more than half the total number, but the regular form, as that of our Fig. 9, corresponding also to the figure given by Sprecher, was not so very frequent, as only one-fifth of them all had this shape. The remaining four-fifths were less regular and showed many transitions between the regular two-ribbed and the regular three-ribbed forms. The characteristic shapes were carefully selected and photographed together; they are shown in Figs. 2-8 at their actual size. Their difference is caused by the inequality in size of the three faces, two remaining nearly always equal, the third diminishing gradually in size; by comparing the Figs. 8 and 2, this diminution of the third side is clearly to be seen on the left side of each figure. In Fig. 2 it is so small that this seed approximates to an irregular two-angled one, the



angle between the lower two ridges of Fig. 2 lying also between  $135^{\circ}$  and  $160^{\circ}$ . Even in seeds like those resembling our Fig. 8 three different forms might be distinguished, although the differences were again not of much importance. A number of them (ten out of nineteen) agreed with the figure, in which we see one face occupying one-half of the stone, the two other the remainder. In seven specimens, however, the two large sides were of equal size, and in the last case (two seeds) the third rib was not entirely developed, as it died out between the base and the apex.

Besides these two- and three-angled forms there were a few four-sided ones, only five in number, four of which are represented in our Figs. 10-13. The seeds represented in Figs. 10 and 11 might be regarded as normal two-angled ones (like our Fig. 1) with two accessory ridges on one of the faces. The stone of Fig. 12, on the other hand, looked much more like a nearly regular three-sided one, with a fourth rib on its largest face.

Fig. 13 was the finest four-angled seed of all, but it was not regular in shape; two of the faces (the lower and the left-hand ones in the figure) were equally large, occupying each one-fourth of the circumference, but in the two others one face was larger and the other smaller than that fourth part.

The fifth and last stone, not represented, resembled both Fig. 10 and Fig. 6, the difference from the latter consisting only in the presence of a fourth ridge on the smallest of its three sides.

The number of the seeds available was evidently too small to permit any general conclusion, but, on the other hand, as it was large enough to give at least an idea as to their frequency, we append the following table:

| <i>Number of<br/>ribs.</i> | <i>Number of<br/>seeds.</i> | <i>Number of seeds<br/>of each kind.</i>  |
|----------------------------|-----------------------------|---|
| 2                          | 47                          | 47 (Fig. 1)<br>6 (Fig. 2)<br>2 (Fig. 3)<br>5 (Fig. 4)<br>9 (Fig. 5)<br>8 (Fig. 6)<br>3 (Fig. 7)<br>19 (Fig. 8)<br>13 (Fig. 9) |
| 3                          | 65                          | 2 (Fig. 10)<br>1 (Fig. 11)<br>1 (Fig. 12)<br>1 (Fig. 13)  |
| 4                          | 5                           |   |
| Total                      |                             | 117   |

Thus the two-sided seeds did not dominate at all, as there were only 40 per cent. of them; there were even more three-angled ones, i. e. no less than 55 per cent., so that it is hardly possible to conclude anything about their ordinary feature. We can therefore agree entirely with the conclusion of Oliver and Salisbury (9, p. 44), who say: 'The facts seem to indicate that, whilst the terms "radiospermic" and "platyspermic" have a definite

use as morphological distinctions, our attitude towards them as criteria of taxonomic importance may require readjustment.'

The differences in number of ribs on the integument and sclerotesta, respectively, of seeds of one and the same species are also recorded in phytopalaeontological literature regarding the seeds of *Physostoma* and of *Trigonocarpus*.

As to *Physostoma elegans*, Oliver mentions (8, p. 83) that in the fifty or so specimens that came under observation the number of ribs varied from nine to twelve.

There is no mention made of the occurrence of transitional forms between them, like those we describe for our *Ginkgo* and which were so frequent, so that our case may perhaps differ in kind from that of *Physostoma*.

Salisbury speaks about the seeds of *Trigonocarpus* (10, p. 68) relatively to the accessory ribs occurring sometimes on the separate valves, and mostly under vascular bundles of the sarcotesta. Since in *Ginkgo*, however, no valves occur—the stony coat lacking fissures at the place of the ribs—and as vascular bundles are absent from the sarcotesta, those seeds cannot, as it seems to us, be compared with the seeds here described.

In the Botanic Garden at Leyden, several *Ginkgo* trees are in cultivation; one of them, the largest and a very beautiful tree, may be a male one, although it has never been known to flower. Another one, much smaller and of regular conical form, produced seeds regularly during recent years, but probably they were not fertilized, as we never succeeded in making them germinate. They were also smaller and had a less developed stony coat than the seeds described here; these latter were probably fertilized, as it is reported that in former years young seedlings have been found under the tree at Slikkerveer.

#### SUMMARY.

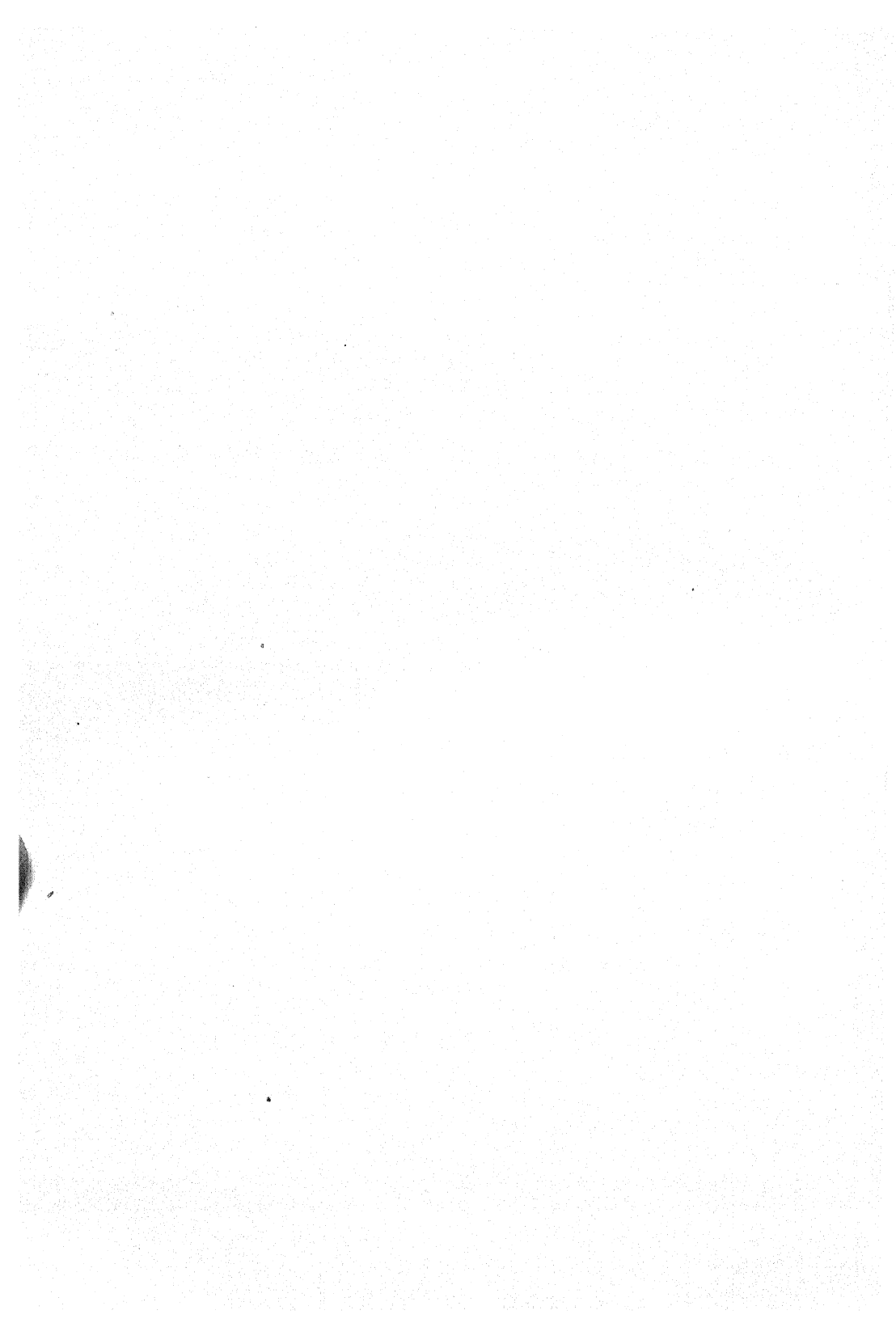
The stony coats of the seeds of *Ginkgo* examined, gathered from a single tree, showed two, three, or four ribs, and they offered at the same time many gradual transitions between the different forms.

BOTANICAL LABORATORY,  
UNIVERSITY, LEYDEN.  
March, 1915.

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# On the Occurrence of Binucleate and Multinucleate Cells in Growing Tissues.

BY

RUDOLF BEER

AND

AGNES ARBER.

A CONSIDERABLE literature exists concerning multinucleate cells in the higher plants, tending to show that such cells are far from rare. For some time we have been making a study of the subject, at first independently and more recently conjointly. Our observations have led us to the conclusion that, in the case of the cortical and medullary parenchyma of stems, a stage in which each cell characteristically contains more than one nucleus often intervenes *as a normal phase of development* between the meristematic and mature conditions. This stage may be highly protracted, or it may be so brief that it is easily overlooked. It is most usual to find two nuclei, but the number may be much higher in certain species. We have not yet satisfied ourselves as to the fate of these nuclei, but there are indications that, at least in some cases, fusions occur at a later stage.

Our present observations would not justify us in saying that the binucleate or multinucleate phase is universal, but we have found it so widely that we should not be surprised if it eventually proved to be the rule rather than the exception. Our preliminary investigation has established the existence of a binucleate or multinucleate phase, of greater or less completeness, in the stem organs of fifty species of Dicotyledons belonging to twenty-seven natural orders and of seventeen species of Monocotyledons belonging to four orders. These cases range from trees to small annual herbs and include examples both of vegetative and reproductive axes. We have at present given most of our attention to stems, but we have also noticed multinucleate cells in the leaf-sheaths of seven species of Gramineae<sup>1</sup> and one species of Araceae, and binucleate cells in the

<sup>1</sup> Multinucleate cells in the stems and leaf-sheaths of grasses were recorded by one of us in 1899. Beer, R.: On the Multinuclear Cells of some Grasses. Natural Science, vol. xv, pp. 434-9, 2 pl., 1899.

cotyledons of four species of Liliaceae and in the roots of *Bambusa*, sp., *Anthurium violaceum*, and *Stratiotes aloides*.<sup>1</sup> Our thanks are due to Miss Ethel Sargent, who has allowed us to examine in this connexion certain of her preparations of Liliaceae seedlings. We have made few observations outside the Angiosperms, but we have found a binucleate stage in the stems of *Araucaria imbricata* and of *Equisetum maximum* and *E. limosum*, showing that this phase is not confined to the highest groups. Taking all the cases together and including stems and leaves, we have found the binucleate or multinucleate phase in seventy-six species belonging to thirty-three orders.

The nuclei of the multinucleate cells generally arise by mitosis, but there are certain exceptional features connected with this mitosis and with the behaviour of the associated cytoplasm. The most striking of these is that two daughter-nuclei in the telophase, between which no wall-formation is in progress, are often found enclosed in a hollow sphere of dense and deeply-staining protoplasm, the appearance at first glance suggesting a cell within a cell. We have observed this singular phenomenon in thirty-five species, representing seventeen natural orders. These and other matters arising out of our observations we hope to discuss in a future paper, but as the subject is a wide one and will take considerable time to work out in detail, we wish to put on record this preliminary survey of our results.

The expenses of our work are being partially borne by a grant from the Newnham College Fellowship Committee, for which we desire to tender our thanks. We also wish to express our indebtedness to Prof. W. Bateson, F.R.S., for his kind permission to grow and collect material at the John Innes Horticultural Institution, and to Mr. R. I. Lynch, M.A., Curator of the Cambridge Botanic Garden, and Mr. E. J. Allard for valuable help in the same connexion.

<sup>1</sup> The binucleate cells in the roots of *Stratiotes* and *Anthurium* appear to differ from the other cases recorded in this note in that the plurality of nuclei here arises through a form of amitosis. See Arber, A.: On Root Development in *Stratiotes aloides*, L., with special reference to the occurrence of Amitosis in an embryonic tissue. Proc. Camb. Phil. Soc., vol. xvii, pp. 369-79, 2 pl., 1914.

# Notes on the Occurrence of Multinucleate Cells.

BY

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With eight Figures in the Text.

THE vegetable cell is generally considered uninucleate. Exceptions to this rule are known either in the case of cells of unusual size, e.g. latex tubules and some algal cells, or cells of very special function, such as embryo sacs, pollen tubes, some jacket (4) and tapetal cells (3); and to these must be added certain cases of multinucleate cells occurring in individual plants, i.e. those recorded by Beer for grasses (2), Arber and McLean for several aquatics, (1) and (6). The purpose of the present communication is to record the occurrence of more than one nucleus, not in highly specialized cells, or those of a particular group of plants, but in different tissues of various immature vegetative organs. The details are given in the following table, which also shows that the plants studied are widely separated in habit, habitat, and systematic position :

| <i>Plant.</i>                            | <i>Organ.</i>                                | <i>Tissue.</i>       |
|--|--|----------------------|
| <i>Pteridium aquilinum</i> . . . . .     | petiole                                      | ground tissue        |
| <i>Marattia</i> sp. . . . .              | "  | "                    |
| <i>Ophioglossum vulgatum</i> . . . . .   | sporangophore                                | "                    |
| <i>Potamogeton crispus</i> . . . . .     | vegetative branch                            | cortex               |
| <i>Sagittaria sagittifolia</i> . . . . . | petiole                                      | ground tissue        |
| <i>Sagittaria lancifolia</i> . . . . .   | petiole                                      | ground tissue        |
| <i>Hydrocharis Morsus-ranae</i>          | bud  | cortex and epidermis |
| <i>Avena sativa</i> . . . . .            | coleoptile                                   | ground tissue        |
| <i>Zizania aquatica</i> . . . . .        | { coleoptile<br>mesocotyl<br>plumular leaves | "                    |
|  | petiole                                      | mesophyll            |
| <i>Arum maculatum</i> . . . . .          |  | ground tissue        |
| <i>Calla palustris</i> . . . . .         | "  | "                    |
| <i>Orontium aquaticum</i> . . . . .      | inflorescence axis                           | "                    |
| <i>Carpinus Betulus</i> . . . . .        | hypocotyl                                    | pith and cortex      |
| <i>Ostrya vulgaris</i> . . . . .         | "  | "                    |
| <i>Corylus Avellana</i> . . . . .        | "  | "                    |
| <i>Fagus sylvatica</i> . . . . .         | "  | "                    |
| <i>Morus nigra</i> . . . . .             | bud  | "                    |
| <i>Polygonum aviculare</i> . . . . .     | inflorescence axis                           | "                    |
| " <i>Persicaria</i> . . . . .            | "  | "                    |
| " <i>amphibium</i> . . . . .             | "  | "                    |
|  | { vegetative stem<br>petiole                 | "                    |
| " <i>orientale</i> . . . . .             | cotyledonary node                            | cortex               |
|  |  | pith                 |

| <i>Plant.</i>                 | <i>Organ.</i>        | <i>Tissue.</i>  |
|-------------------------------|----------------------|-----------------|
| <i>Nuphar</i> sp. . . . .     | peduncle             | ground tissue   |
| <i>Anona</i> sp. . . . .      | cotyledonary node    | cortex          |
| <i>Ribes sanguineum</i> . . . | { petiole            | "               |
|                               | { inflorescence axis | pith            |
| <i>Vicia Faba</i> . . . . .   | seedling stem        | pith and cortex |
| <i>Linum usitatissimum</i> .  | plumule              | pith            |
| <i>Acer Pseudo-Platanus</i> . | petiole              | ground tissue   |
| <i>Aesculus Hippocastanum</i> | bud                  | pith and cortex |
| <i>Tilia europaea</i> . . . . | "                    | "               |
| <i>Hedera Helix</i> . . . . . | petiole              | ground tissue   |
| <i>Hottonia palustris</i> . . | { bud                | cortex          |
|                               | { inflorescence axis | "               |
| <i>Fraxinus excelsior</i> . . | "                    | cortex and pith |
| <i>Syringa vulgaris</i> . . . | petiole              | cortex          |
| <i>Limnanthemum peltatum</i>  | "                    | "               |
| <i>Cucurbita Pepo</i> . . . . | hypocotyl            | "               |
| <i>Helianthus annuus</i> . .  | "                    | cortex and pith |

In all the above instances the two nuclei were in the same focal plane, cases where they were not so being regarded as possibly due to the effect produced by the superposition of cells. It must, however, be remembered that binucleate cells may be unrecognized by the fact that only one of the nuclei is included in the plane of the section, and this consideration is of greater importance the higher the number of nuclei in the cell, for the less likely will they be all to occur in the same plane (cf. Fig. 7). Nevertheless, trinucleate cells have been recognized with practical certainty in several of the above, e. g. *Arum maculatum*, *Limnanthemum peltatum*, *Zizania aquatica*; and in *Morus nigra* (Fig. 1) they are obvious and frequent.

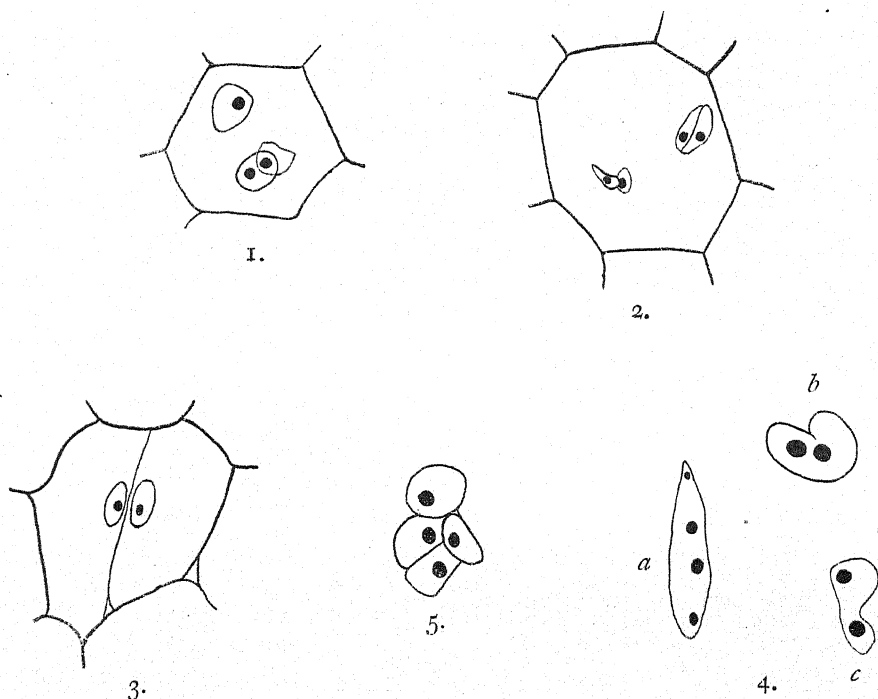
It is very probable that more than three nuclei occur in some cases, and in *Morus nigra* it would be difficult to say sometimes how many nuclei a particular cell did contain, for we find what can best perhaps be described as a nuclear complex (Fig. 5) where a nucleus is dividing into more than two daughter-nuclei, or, which amounts to much the same thing, where the products of the division of a nucleus are themselves dividing before separation.

Judging from the data at present available, the frequency of occurrence of multinucleate cells will be found to vary considerably in different plants. In some cases, such as that of *Ophioglossum vulgatum*, the occurrence is very likely rare and sporadic, but in most of the plants mentioned above a single section in the appropriate region will reveal several, perhaps many instances. The relative frequency of occurrence also varies in different tissues. In my experience, pith is far the most likely tissue, after this cortex, where they are more often found in the bundle or stelar-sheath—a point to which I attach some significance, and which will receive fuller treatment in another connexion. Binucleate cells are probably very rare in the epidermis, and I have not yet seen them in vascular tissue (see, however, Arber (1) and Thompson (7)). With regard to organs, opening buds have so far proved the most prolific in showing multinucleate cells; Angio-



spermic seedlings<sup>1</sup> are also good, and after these, inflorescence axes, petioles, &c.

The binucleate cells and their nuclei do not generally differ in size, shape, &c., from the other cells and nuclei of the tissues in which they occur, so that the usual explanation of coenocytic structures (Haberlandt (5)) cannot apply here. The question further arises as to the origin and fate of



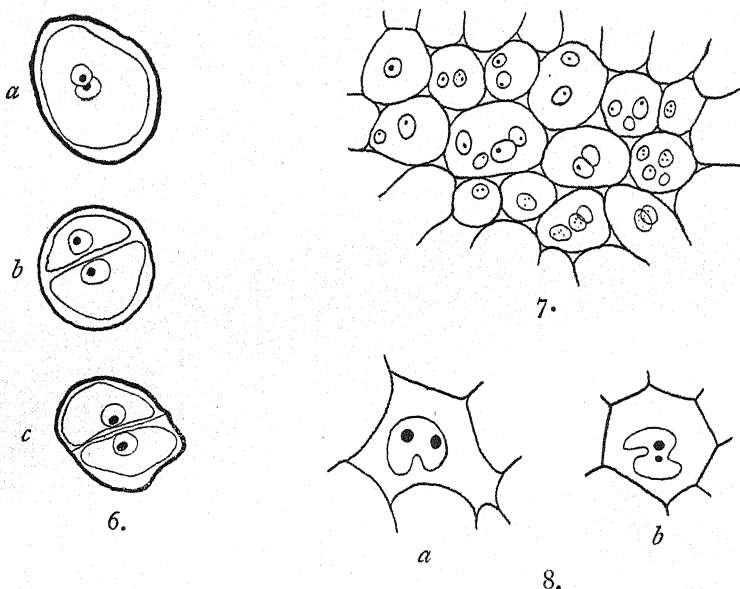
FIGS. 1-5. *Morus nigra*. 1. A trinucleate cell. 2. A multinucleate cell showing probable longitudinal fission of one of the nuclei. 3. Cell which has just divided.  $\times 400$ . 4. Nuclei, *a* spindle-shaped, *b* lobed, *c* constricted.  $\times 930$ . 5. Nuclear complex.  $\times 600$ .

these additional nuclei, i. e. are they produced amitotically, or by the failure of karyokinesis to produce partition walls? And since cells of older tissues are apparently uninucleate, do the extra nuclei abort, does fusion take place, or are walls subsequently formed?

With reference to the first question, the second suggestion is possibly true in certain cases, but in general I think that the multinucleate cells of vegetative tissues are produced by amitosis. In the first place, this is strongly suggested by the position of the nuclei with regard to each other, for they are usually in close contact—a fact difficult to explain

<sup>1</sup> Dr. E. N. Thomas has kindly permitted me to examine a number of her preparations of seedling Gymnosperms, but I was not able to satisfy myself in a single instance of a binucleate cell.

if they are the result of karyokinesis. Stages in their separation from one another can easily be found, and appearances strongly suggestive of amitosis have occasionally been observed (Figs. 2, 4, *b* and *c*, and 8, *b*). The constriction of the nuclei described by Beer for grasses, the longitudinal fission for aquatics by McLean, and the lobing in *Stratiotes* by Arber have all been observed in plants far removed from aquatics in habit, and from grasses in classification; but it must be confessed that such stages are rare, although careful search has been made for them, and in some cases, where they are



FIGS. 6-8. 6. Cells from pith of *Aesculus Hippocastanum*, illustrating probable course of cell division. *a*, binucleate cell; *b*, cell with two nucleated protoplasts; *c*, cell very recently divided.  $\times 400$ . 7. *Zizania aquatica*. Small piece of mesophyll of plumular leaf (from a preparation by Miss Sargent and Dr. A. Arber).  $\times 600$ . 8. Parenchymatous cells from the vascular tissue of *a*, *Adiantum Capillus-Veneris*, and *b*, *Pteris* sp., showing lobed nuclei.  $\times 930$ .

present, binucleate cells have not been seen (*Adiantum* and *Pteris*, Fig. 8). This, however, scarcely militates against amitosis, since karyokinesis is even more rarely found, and the nuclei must be produced one way or the other. If, as seems probable, the actual process of division takes place very quickly, or at a special time, the failure to find stages in amitosis at all frequently is easily explained, and further work is contemplated to throw light on this point.

With regard to the ultimate fate of multinucleate vegetative cells, we may surely dismiss the hypotheses of abortion of all but one nucleus, or fusion, since they are improbable on theoretic grounds, and no stage in either process has been observed.<sup>1</sup> Walls, then, must be formed, though evidently

<sup>1</sup> Contrast Carothers' (3) view as to the course of events in the multinucleate cells of *Ginkgo biloba*.

not immediately after direct division of the nuclei. In several different plants, which show binucleate cells, I have observed two nucleated protoplasts within one cell-wall (Fig. 6, *b*), and though this appearance might conceivably be due to artefact, comparison with other cells of the tissues in which they occur makes it seem probable that this apparent cleavage of the protoplasm represents the first stage of wall-formation.

Finally, it may be noted that multinucleate cells tend to occur in regions of activity (cotyledonary nodes of seedlings) and rapid elongation (stems of buds). In general, the cells show dense cytoplasm, and the nuclei are usually near the centre, possessing one or more large, refringent, and deeply staining nucleoli. Considerable plasticity is often shown in the size and shape of the nuclei in these tissues (Figs. 2 and 4). Binucleate cells in actual meristem occur rarely, if at all; for example, the pith of the bud of *Morus nigra*, fixed February 5, 1915, showed typical uninucleate cells, while the pith towards the apex in opened buds gathered May 9, 1915, was composed mostly of multinucleate cells.

If direct nuclear division with subsequent wall-formation does occur, as the above observations suggest, the 'heretical opinion' expressed by Arber that 'amitosis plays an active part in the growth of the young roots of *Stratiotes*' ((1), p. 374) is not only confirmed, but shown to be of far wider application.

#### SUMMARY.

1. Multinucleate (usually binucleate) cells occur in different tissues of various young organs in a number of plants widely separated both as to habit and systematic position, and it is suggested that their occurrence is characteristic of regions of active growth.

2. In some cases, at least, these nuclei are probably produced by amitosis followed by wall-formation, and the view is maintained that these processes are a means of tissue-formation in rapidly growing organs.

Many of the preparations used in the above were made in connexion with a research I have been carrying out for some years in Professor Oliver's laboratory, University College, London. Microtome series through some grass seedlings were kindly lent me by Miss Sargent and Dr. Agnes Arber, and some other preparations by Dr. E. N. Thomas and several members of her Department at Bedford College, to all of whom I tender my thanks. I am also grateful to Dr. Agnes Arber for the interest she has taken in my work, and to my assistant, Miss G. E. M. Piper, B.Sc., for her sympathetic help.

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Since the above was written I have discovered multinucleate cells in the pith, just above the nodes, of *Polygonum Persicaria*, in older parts of the stem, where these cells are rare or absent in the internodes. Most, if not all, the cells are at least binucleate, and some show as many as five nuclei in a single plane. This is of interest since it affords a striking instance of multinucleate cells existing in a specially active tissue capable of rapid growth, for it is just at the nodes that geotropic curvatures take place in this plant.

## The Root-nodules of *Ceanothus americanus*.

BY

PROFESSOR W. B. BOTTOMLEY.

With Plate XXVIII.

AS far as can be ascertained, Dr. W. J. Beal of the Agricultural College, Michigan, U.S.A., was the first to call attention to the tubercles occurring on the roots of *Ceanothus*. At the meeting of the American Association for the advancement of Science held at Indianapolis in 1890 he exhibited specimens of what he called 'root-galls' on the larger roots of *Ceanothus americanus*.

Two years later Atkinson described the structure of these root-galls and stated that the gall is inhabited by a fungus which 'when mature forms compact botryoid clusters in the affected parenchymatous cells of the gall, the central portion being composed of a complexly-branched mass of threads bearing at their ends on the periphery of the mass the globose sporangia'. He considered the fungus to be related to the genus *Frankia* of Brunchorst, and proposed for it the name *Frankia Ceanothi*.

No further mention of these structures can be found until 1910, when Kellerman, in a paper on 'Nitrogen Gathering Plants', states that 'nitrogen fixing Bacteria, apparently similar to the Bacteria isolated from the Leguminosae, have been isolated from nodules of *Ceanothus*'. Unfortunately he gives neither references to any research work nor experimental evidence in support of his statements. Kellerman's paper is illustrated by photographs of root-nodules from a number of non-leguminous plants, and on examining these one is struck by the remarkable resemblance between the nodules of *Alnus*, *Elaeagnus*, and *Ceanothus*. As Hiltner had demonstrated in 1896 that the root-nodules of *Alnus* and *Elaeagnus* are concerned with nitrogen assimilation, the resemblance suggested the possibility of the root-nodules of *Ceanothus* having a similar physiological significance.

There was a difficulty at first in obtaining material for investigation. An examination of the roots of *Ceanothus* plants growing at Kew Gardens, Chelsea Physic Gardens, and various nurseries and private gardens failed to reveal a single nodule. The foreman gardener of one large nursery

stated that during his fourteen years' experience of cultivating *Ceanothus*, both the deciduous forms belonging to the *C. azureus* group and the small-leaved varieties such as *C. dentatus* and *C. floribundus*, he had never seen a root-nodule in any *Ceanothus* plant similar to those he was familiar with on *Elaeagnus* roots. This is perhaps not surprising when one remembers that *Ceanothus* is an imported plant, and presumably the soil is devoid of the requisite nodule-forming Bacteria. A similar case is known in *Soja* bean plants grown in this country, which fail to produce root-nodules unless inoculated with the specific organism from abroad.

Eventually a plentiful supply of root-nodules was obtained from *Ceanothus* plants growing wild in North America, where the genus is indigenous, through the kindness of Dr. Kellerman of the United States Department of Agriculture and Professor F. Clements of the University of Minneapolis.

Nodule-bearing roots of two different species were received—*Ceanothus americanus* (New Jersey tea) and *Ceanothus velutinus* (mountain balm). As a preliminary examination showed that the nodules of both species are practically identical, the following description of *C. americanus* applies equally to *C. velutinus*.

#### EXTERNAL STRUCTURE OF THE NODULE.

The nodules when young are flesh-coloured, cylindrical outgrowths, from 3 to 8 mm. in length by 1 to 2 mm. broad. They are first visible as tiny lateral swellings on the young roots, and soon attain a breadth of 1 to 2 mm., after which growth is confined to the apex until a length of 6 to 8 mm. is reached. This concludes the growth for the first year. In the following year they continue their growth by branching near the apex, producing two to five branches, and each branch repeats the growth of the primary nodule. This branching is continued in successive years, each year's growth remaining from 1 to 2 mm. in diameter and 4 to 6 mm. in length. Thus the nodules are perennial, and after a few years there is formed a loosely-branched, rotund mass, the size of a small walnut. The number of branches arising each year is variable, two, three, four, or even five branches being produced near together. Hence there is a greater diversity of branching in *Ceanothus* nodules than in other non-leguminous nodules where bi- or trifurcation is characteristic.

#### INTERNAL MORPHOLOGY.

The material used for the purpose of investigating the internal structure of the nodules was fixed in either Bouin's fixative or alcohol, and microtomed sections were stained with Flemming's triple stain, Heidenhain's iron-haematoxylin, or Kiskalt's amyl gram. A transverse section through

the centre of a young nodule or branch shows that the relation of the different tissue systems differs but little from that of a normal root. There is a central stele surrounded by an abnormal development of cortical cells and a protective corky layer on the outside. The xylem forms a solid mass in the centre of the stele. As all the elements are small it is difficult to distinguish the separate protoxylem groups, but where determined they appear to vary in number from three to five in different nodules. The xylem is surrounded by several layers of parenchymatous cells, the inner layers consisting chiefly of elongated cells (phloem), and the two outer layers of iso-diametric cells (pericycle).

When the nodule branches, secondary thickening occurs in the primary stele, whereby a certain amount of secondary xylem is produced each year, thus forming a strong supporting stalk for the annually increasing cluster of branches. The stele is bounded by a very definite endodermis, which, as in the nodules of *Alnus*, *Elaeagnus*, and *Myrica*, is characterized by the cells being filled with reserve material, chiefly proteid and oil drops.

Outside the endodermis the cortical cells are arranged in two zones—a narrow inner zone of small cells, always free from Bacteria, and an outer broad zone of large cells, most of which are elongated radially, and containing much enlarged cells crowded with Bacteria. This outer zone is enclosed by a layer or layers of suberized cells.

A median longitudinal section shows that the young nodule is surrounded by a protective layer of suberized cells. In certain localized areas these cells occur in relatively large masses. This is seen especially at the apex of the nodule, where they function as a kind of root-cap.

In the older nodules and branches a definite phellogen is present, which produces a few very regular layers of periderm, the cells of which always contain a dark brown substance.

The apex of the young nodule, below the protective layer, is occupied by a group of meristematic cells which covers the end of the stele. Immediately below this meristematic tissue many of the cortical cells are infected with rod-shaped Bacteria, readily seen *in situ* in sections cut from material fixed in alcohol and stained with Kiskalt's amylogram stain. The organisms occupy at first only a small portion of the cytoplasm of the cell. Here, however, they actively divide, and a little further from the apex of the nodule the infected cells are seen to have increased in size and are filled with Bacteria. Lower down the nodule these cells become still more enlarged and the rod-shaped Bacteria are replaced by large spherical bodies containing either two or four denser portions. These are evidently the 'globose sporangia' of Atkinson.

Towards the base of the nodule the infected cells gradually lose their contents, and are finally seen as dead empty cells.

## ORIGIN AND BRANCHING OF THE NODULES.

From the material available it was not possible to trace the earliest stages in the formation of the nodule, but sections of young nodules indicate very clearly that they are modified lateral roots.

They consist of an apical meristematic region below which is a branch from the root-stele surrounded by cortical tissue containing many infected cells, the endodermis of the nodule-stele being continuous with that of the root-stele. Presumably certain cortical cells of the root became infected by Bacteria as in the formation of leguminous nodules, and these stimulate the formation of a lateral root at this point. This lateral root never breaks through the cortex, but as it extends into the infected area the outer cortical cells above this area become meristematic, and by their subsequent growth a long cylindrical nodule is formed instead of a typical lateral root.

The branches of the primary nodule are very evidently endogenous in origin. They arise from an active development of the pericycle cells opposite a protoxylem group. A stelar branch is thus formed, and as this extends into the cortex an apical mass of meristematic cells becomes differentiated which soon produces a visible branch to the nodule.

This method of branching is quite different from that which is characteristic of the nodules of *Alnus*, *Elaeagnus*, and *Cycas* where there is a di- or trichotomy of the apical meristem. In *Ceanothus* as in *Myrica* the branches are primarily due to the formation of stelar outgrowths, the essential difference between the two being that in *Ceanothus* the stele never emerges from the nodule, and the apex remains meristematic, continuing its growth each year, whilst in *Myrica* the stele in its second year pushes through the apex of the nodule and forms a small hair-like rootlet, thus preventing further apical growth.

## ISOLATION AND CULTIVATION OF THE BACTERIA.

As described above, two different structures are found in the infected cells, rod-shaped organisms near the apex of the nodule and spherical bodies lower down. The rod-shaped organisms when isolated by the methods described in detail for *Myrica* nodules (Ann. Bot., xxvi, p. 114) appear to be identical with the *Bacillus radicola* group found in leguminous nodules, giving the characteristic staining reaction with Kiskalt's amyl gram, and typical colonies when grown on nutrient agar. The spherical bodies are very similar in appearance to the 'coccus' forms described by Spratt in *Alnus* and *Elaeagnus* nodules. When grown in ordinary *B. radicola* culture solution they soon lose their spherical condition and break up into as many typical rod-shaped forms as there are dense portions present. The reverse change from rods to spherical bodies can be seen in old colonies



grown on agar medium. These spherical bodies therefore are not 'sporangia' as Atkinson interpreted them, but are similar to the 'bacteroids' found in leguminous nodules which are known to be a resting and resistant form of the active bacillus produced by unfavourable environmental or nutritive conditions. In *Ceanothus* the alteration in conditions which must necessarily occur in the host cell as it becomes depleted of food and crowded with Bacteria causes the organism to assume the 'bacteroid' form which in this case is spherical instead of the Y- or V-shape of the leguminous nodule.

#### FIXATION OF ATMOSPHERIC NITROGEN.

To test the possibility of these organisms being concerned with the fixation of atmospheric nitrogen pure cultures from each species of *Ceanothus* were grown in Erlenmeyer flasks in the usual non-nitrogenous culture solution, one set of flasks being autoclaved immediately after inoculation to serve as controls. The flasks were incubated at 26°C. for seven days, during which time the contents of the control flasks remained clear, whilst the non-sterilized flasks became cloudy. Kjeldahl nitrogen determinations of the contents of the flasks gave the following average figures:

|                      | N. in control. | N. in culture. | N. gain per 100 c.c.<br>of culture. |
|----------------------|----------------|----------------|-------------------------------------|
| <i>C. americanus</i> | 0.24 mg.       | 2.57 mg.       | 2.33 mg.                            |
| <i>C. velutinus</i>  | 0.14 "         | 2.36 "         | 2.22 "                              |

This gain in nitrogen is very similar in amount to that obtained from cultures of the Bacteria in the root-nodules of *Alnus*, *Elaeagnus*, and *Myrica*. It is therefore evident that the root-nodules of *Ceanothus* are definitely concerned with nitrogen assimilation.

#### SUMMARY.

1. The root-nodules of *Ceanothus americanus* are modified lateral roots.
2. They are perennial and increase in size each year by the formation of endogenous outgrowths (branches) similar in structure to the primary branch.
3. Each primary nodule and branch when fully grown shows four zones: (a) an apical meristematic zone; (b) an infection zone, where the cortical cells are becoming infected with Bacteria; (c) a bacterial zone, containing many radially-elongated enlarged cells filled with Bacteria; (d) a basal zone, almost free from bacterial cells.
4. The younger bacterial cells contain rod-shaped organisms, the older ones spherical bodies. These latter are the 'bacteroid' condition of the active nitrogen-fixing rod-shaped bacillus.

5. The Bacteria, when isolated and grown in pure culture, can fix free atmospheric nitrogen, and from their structure, mode of growth and formation of 'bacteroids' evidently belong to the *Bacillus radiculicola* group.

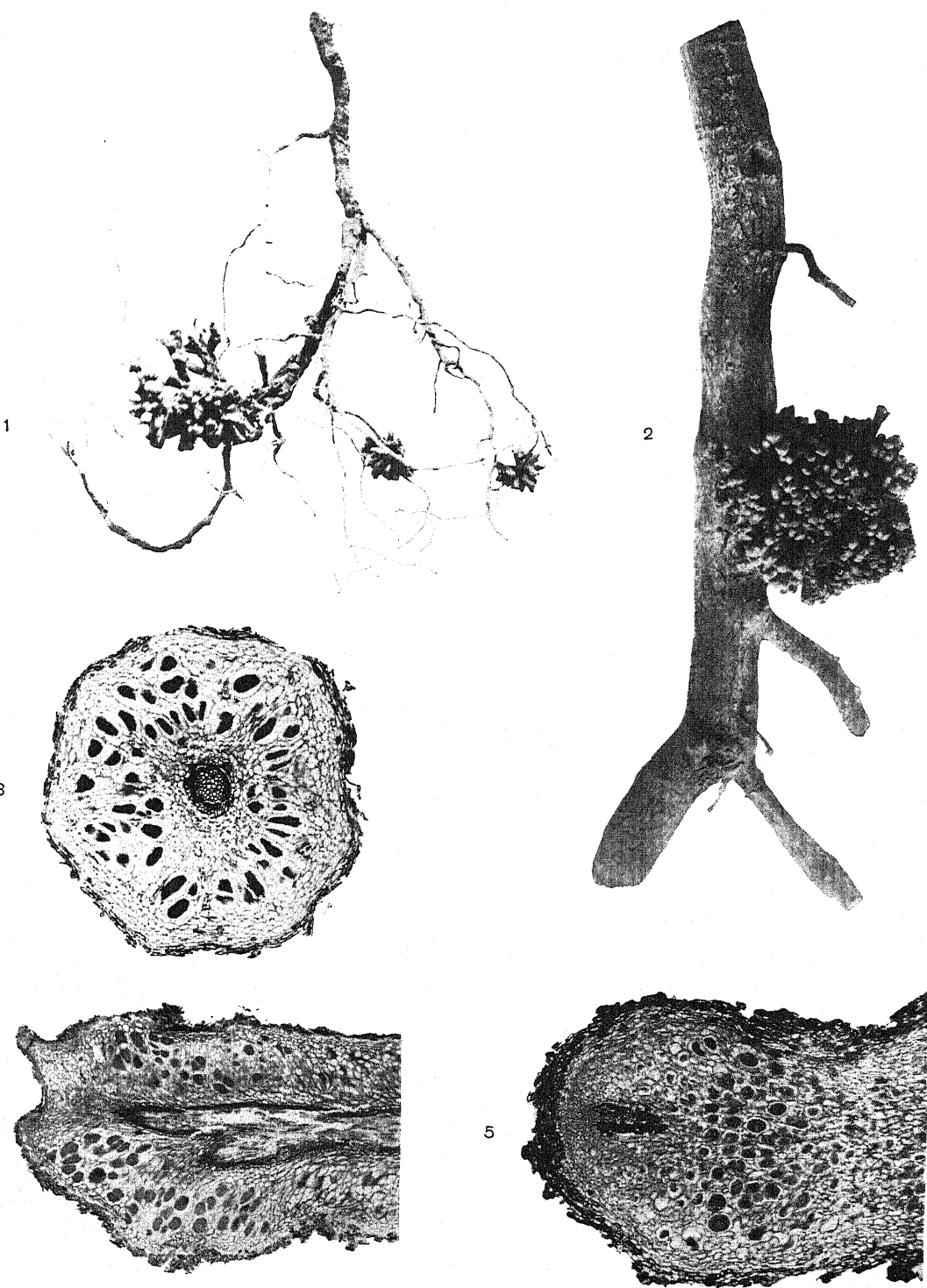
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EXPLANATION OF PLATE XXVIII.

- Fig. 1. *Ceanothus velutinus*. Root-nodules. Nat. size.  
 Fig. 2. *Ceanothus americanus*. Root-nodules. Nat. size.  
 Fig. 3. *C. americanus*. Transverse section of root-nodule, showing central stele and bacterial zone with radially elongated cells filled with Bacteria.  $\times 50$ .  
 Fig. 4. *C. americanus*. Longitudinal section of root-nodule, showing endogenous origin of branch.  $\times 30$ .  
 Fig. 5. *C. americanus*. Tangential section of root-nodule, showing the four zones.  $\times 50$ .



BOTTOMLEY — CEANOTHUS.

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## Studies in Permeability.

### II. The Effect of Temperature on the Permeability of Plant Cells to the Hydrogen Ion.

BY

WALTER STILES

AND

INGVAR JØRGENSEN.

With four Diagrams in the Text.

IN the first of these papers we have indicated that the cells of potato tuber absorb hydrogen ions very rapidly, and in the succeeding article of this series it will be shown that this rapid absorption is a general characteristic of acids. It becomes of interest to discover whether this absorption is due to simple diffusion into the cell, whether it is due to adsorption, or whether the entrance of the acid is the result of its chemical combination with some substance or substances of the cell, for all information of this kind is likely to be of help in the elucidation of the mechanism of permeability. We have therefore examined the influence of temperature on the absorption of hydrochloric acid by potato cells, as these three processes, diffusion, adsorption, and chemical action, should all be influenced differently by alterations in temperature.

#### METHOD.

The method of investigation has been briefly indicated in a previous paper, here we may give further details.

The tissue used consisted of discs of potato tuber which were 1 cm. in diameter and weighed about 0.5 gm. They were washed in a gentle stream of tap water for about an hour, then rinsed in distilled water, and transferred to the acid solution.

The acid used was hydrochloric acid, made up by means of specific gravity tables to a strength of about  $\frac{N}{10}$ , which was diluted for use to 0.001 N. Subsequent titration with standard alkali showed that the stock

acid solution was actually 0.110 N, so that the acid used in the experiments had a concentration of 0.0011 N. This low concentration was used as it is unlikely to damage the plant-cells for some time; a higher strength of acid is likely to be more dangerous in this regard.

The experiments were carried out in stoppered bottles. In each bottle was placed 100 c.c. of acid and 20 discs of potato. A number of such bottles were placed in a thermostat kept at the desired temperature, and at

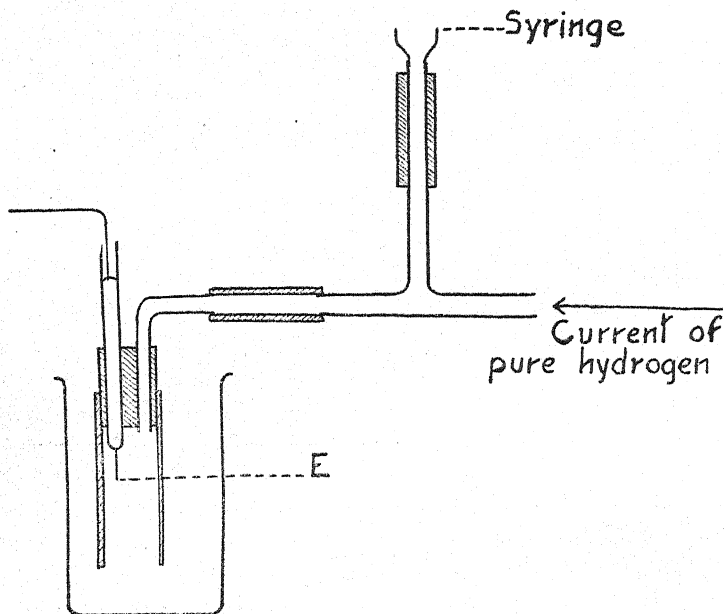


FIG. 1. This shows the form of hydrogen electrode used. The point electrode is shown at E. The liquid whose acidity is to be determined is placed in the larger vessel. Hydrogen is passed through the electrode vessel for a few seconds. By moving the plunger of the syringe backwards and forwards a few times while the gas is passing, the last traces of air are removed from the apparatus. The current of gas is stopped, and by pushing the plunger of the syringe in and out the electrode is wetted with the liquid. Finally the liquid is brought to such a level that the point just touches it. Equilibrium is very quickly attained with an electrode in good working condition.

various intervals of time the bottles were removed, usually in duplicate, the acid poured off, and the concentration of the acid solution measured.

In order to measure the acidity of solutions in this dilution, into which moreover various organic substances may have diffused out from the cell-tissue, the ordinary titration methods are useless. The measurement has therefore been made by means of the hydrogen electrode.

When a metal is immersed in a solution of one of its salts, an electromotive force is set up at the surface of contact of the metal and salt, and this E.M.F. is dependent upon the concentration of the metal ion in the

solution of the salt. The same is true also for an electrode of hydrogen immersed in an acid, i. e. a solution containing hydrogen ions.

As hydrogen electrode we used a modification of that suggested by Walpole (Bioch. Journ. 1913). The electrode vessel consisted of a small piece of glass tubing closed at one end with a rubber stopper. Through this stopper passed a glass tube with the electrode of platinized platinum wire sealed into the lower end, and a piece of capillary glass tubing connected to a T-piece. The other two ways of the T-piece were connected one to a small glass syringe, the other to an apparatus for generating hydrogen. This was carefully purified before passing into the electrode vessel.

The electrode vessel could be placed in a larger vessel containing the liquid whose acidity was to be determined (Fig. 1).

The electrode was charged in the usual manner as described by Walpole.

The hydrogen electrode was combined with an  $\frac{N}{10}$  KCl-Calomel electrode, a 3.5 N solution of KCl being used as intermediate liquid. Kahlbaum's pure potassium chloride with certificate of guarantee was used and the mercury and mercurous chloride were carefully purified. The form of the calomel electrode is shown in the accompanying sketch (Fig. 2). The glass vessel itself is filled above the mercury and calomel with  $\frac{N}{10}$

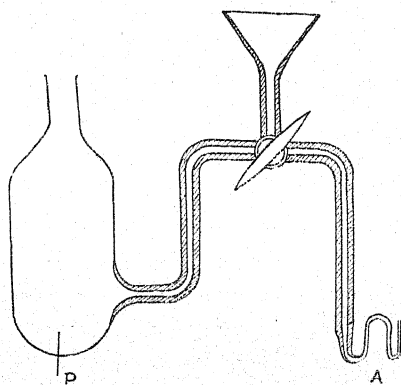


FIG. 2. The diagram shows the form of Calomel electrode used. The bent part at A actually lies in a plane at right angles to the rest of the apparatus. The platinum point P dips into a mercury cup.

KCl saturated with calomel, and this solution occupies the tube between the vessel itself and the three-way tap. The intermediate liquid of 3.5 N KCl occupies the rest of the tube, and can be run out by turning the tap, or renewed from the same funnel. The tap is ungreased and kept closed, there always being enough liquid held by capillarity to ensure sufficient conduction.

The electromotive forces manifested by this combination were compared with a Weston Normal Cell manufactured by the Cambridge Scientific Instrument Company. The standard cell and the combination of calomel electrode and hydrogen electrode were compared with a 2-volt accumulator by Poggendorf's method. A capillary electrometer (enclosed pattern) was first used as a null instrument, but this was afterwards replaced by a delicate moving coil galvanometer manufactured by

R. W. Paul, and which proved a much more convenient and sensitive arrangement.

The hydrogen-ion concentrations of the various solutions were then calculated according to the formula

$$e_1 - e_0 = 2.3026 \frac{RT}{F} \log \frac{C_0}{C_1}$$

where  $e_0$  is the E.M.F. given with a liquid of hydrogen-ion concentration  $C_0$  and  $e_1$  the E.M.F. given with a liquid of hydrogen-ion concentration  $C_1$ .  $R$  is the gas constant,  $T$  the absolute temperature, and  $F$  signifies 1 faraday (= 96580 coulombs).

That the apparatus was giving correct readings was tested by the use of different concentrations of acids of known strength.

### THE RESULTS.

Experiments were conducted at four different temperatures, 0°C., 10°C., 20°C., 30°C. The amounts of acid absorbed by the tissue after different lengths of time at these various temperatures are indicated in the following tables. The second column shows the increase of the E.M.F. at the surface of the hydrogen electrode in each of the acid solutions as compared with that in the case of the original 0.0011 N acid. The third column shows the concentration of the acid as calculated from this E.M.F., and the next column the actual absorption. The numbers in the last column we shall refer to later.

#### *Temp. 0°C.*

| <i>Time in hours.</i> | <i>Increase of<br/>E.M.F. in volts.</i> | <i>Relative Concentration<br/>(C) of Hydrogen Ion<br/>in Solution.</i> | <i>Relative quantity<br/>of Hydrogen Ion<br/>absorbed.</i> | <i>— Log. C.</i> |
|-----------------------|---|--|--|------------------|
| 0.0                   | 0.0                                     | 1.000  | 0.0  | 0.0              |
| 3.0                   | 0.0069                                  | 0.751  | 0.249  | 0.124            |
| 6.0                   | 0.0120                                  | 0.610  | 0.390  | 0.215            |
| 8.0                   | 0.0172                                  | 0.493  | 0.507  | 0.307            |
| 8.0                   | 0.0173                                  | 0.491  | 0.509  | 0.309            |

#### *Temp. 10°C.*

| <i>Time in hours.</i> | <i>Increase of<br/>E.M.F. in volts.</i> | <i>Relative Concentration<br/>(C) of Hydrogen Ion<br/>in Solution.</i> | <i>Relative quantity<br/>of Hydrogen Ion<br/>absorbed.</i> | <i>— Log. C.</i> |
|-----------------------|---|--|--|------------------|
| 0.0                   | 0.0                                     | 1.000  | 0.0  | 0.0              |
| 0.5                   | 0.0035                                  | 0.870  | 0.130  | 0.060            |
| 0.5                   | 0.0027                                  | 0.868  | 0.102  | 0.048            |
| 1.0                   | 0.0062                                  | 0.781  | 0.219  | 0.107            |
| 2.0                   | 0.0084                                  | 0.709  | 0.291  | 0.149            |
| 2.0                   | 0.0084                                  | 0.709  | 0.291  | 0.149            |
| 2.5                   | 0.0145                                  | 0.561  | 0.439  | 0.251            |
| 4.0                   | 0.0180                                  | 0.488  | 0.512  | 0.312            |
| 5.0                   | 0.0231                                  | 0.399  | 0.601  | 0.399            |
| 5.0                   | 0.0202                                  | 0.447  | 0.553  | 0.350            |
| 6.0                   | 0.0256                                  | 0.361  | 0.639  | 0.443            |
| 6.0                   | 0.0249                                  | 0.370  | 0.630  | 0.432            |



Temp. 20°C.

| Time in hours. | Increase of<br>E.M.F. in volts. | Relative Concentration<br>(C) of Hydrogen Ion<br>in Solution. | Relative quantity<br>of Hydrogen Ion<br>absorbed. | —Log. C. |
|----------------|---------------------------------|---|---|----------|
| 0.0            | 0.0                             | 1.000   | 0.0   | 0.0      |
| 1.0            | 0.0105                          | 0.649   | 0.351   | 0.188    |
| 1.0            | 0.0117                          | 0.619   | 0.381   | 0.208    |
| 2.0            | 0.0205                          | 0.430   | 0.570   | 0.366    |
| 2.0            | 0.0219                          | 0.407   | 0.593   | 0.390    |
| 3.0            | 0.0276                          | 0.321   | 0.679   | 0.494    |
| 3.0            | 0.0269                          | 0.331   | 0.669   | 0.480    |
| 4.0            | 0.0399                          | 0.194   | 0.806   | 0.712    |
| 4.0            | 0.0421                          | 0.177   | 0.823   | 0.752    |
| 5.0            | 0.0442                          | 0.162   | 0.838   | 0.792    |
| 5.0            | 0.0513                          | 0.121   | 0.879   | 0.917    |

Temp. 30°C.

| Time in hours. | Increase of<br>E.M.F. in volts. | Relative Concentration<br>(C) of Hydrogen Ion<br>in Solution. | Relative quantity<br>of Hydrogen Ion<br>absorbed. | —Log. C. |
|----------------|---------------------------------|---|---|----------|
| 0.0            | 0.0                             | 1.000   | 0.0   | 0.0      |
| 0.5            | 0.0102                          | 0.667   | 0.333   | 0.176    |
| 0.5            | 0.0140                          | 0.581   | 0.419   | 0.236    |
| 1.0            | 0.0232                          | 0.398   | 0.602   | 0.400    |
| 1.0            | 0.0200                          | 0.417   | 0.583   | 0.380    |
| 2.0            | 0.0418                          | 0.190   | 0.810   | 0.721    |
| 2.0            | 0.0425                          | 0.185   | 0.815   | 0.733    |
| 3.0            | 0.0660                          | 0.0770  | 0.923   | 1.114    |
| 3.0            | 0.0720                          | 0.0751  | 0.925   | 1.124    |

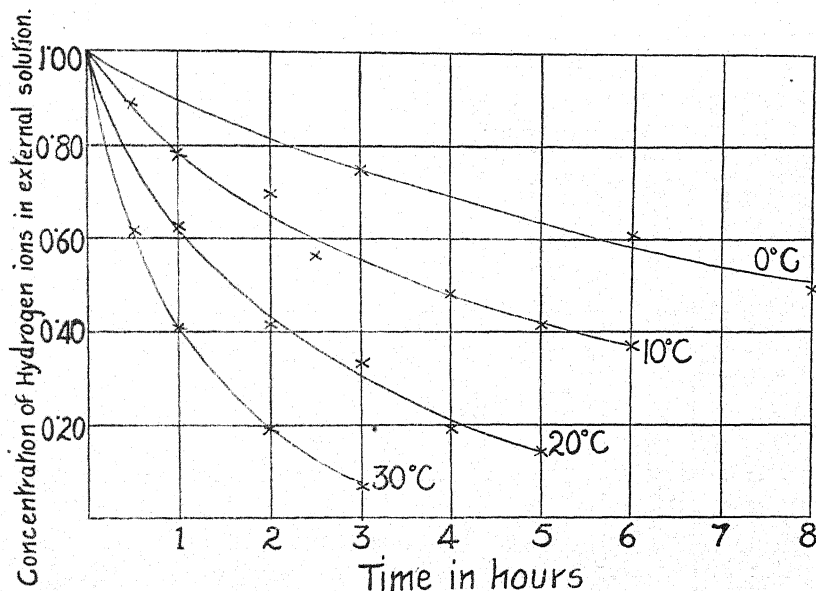


FIG. 3. For explanation see text.

The curves shown in Fig. 3 exhibit graphically the relation between the time the absorption has proceeded and the quantity of acid left in the solution at that time. All the curves strongly resemble exponential

curves in shape, and this is confirmed by plotting curves between the time and the logarithm of the concentration (see last column of tables and Fig. 4).

The relation between time and concentration is then given by the equation

$$-\log C = kt + k' \quad . \quad . \quad . \quad . \quad (1)$$

and if  $x$  represents the amount of acid absorbed at any time we have

$$-\log (A - x) = kt + k'$$

where  $A$  is the original quantity of acid present.

If this is taken as unity we have  $k' = 0$ , and  $k = \frac{-\log (A - x)}{t}$ .

The rate of absorption  $\frac{dx}{dt}$  is then given by the equation

$$\frac{dx}{dt} = k(A - x) \quad . \quad . \quad . \quad . \quad (2)$$

i. e. the rate of absorption is proportional to the concentration at any time and to the constant  $k$ , and  $k$  has the same value in equation (2) as in equation (1).

From measurements of the curves obtained from experiments and shown in Fig. 4 we have the following values for  $k$  at different temperatures :

| <i>Temperature.</i> | <i>k</i> |
|---------------------|----------|
| 0°                  | 0.036    |
| 10°                 | 0.081    |
| 20°                 | 0.174    |
| 30°                 | 0.380    |

i. e. the rate of absorption is increased by a rise of 10°C. as follows :

|                |            |
|----------------|------------|
| from 0° to 10° | 2.22 times |
| „ 10° „ 20°    | 2.17 „     |
| „ 20° „ 30°    | 2.18 „     |

The rate of absorption is therefore increased by about 2.2 times for a rise of 10°C.

Now if the absorption of the hydrogen ion were controlled by simple diffusion into the cell-tissue, one would not expect the increase in its rate of entrance to be of this order. Rather an increase in much lower proportion would be expected, of about the order of 1 : 3.

As regards the effect of temperature on the rate of adsorption, the coefficient seems to be of the same order as that of diffusion ; certainly the van't Hoff rule is not followed.

But it has been shown by van't Hoff and subsequent workers that the rate of many chemical reactions is doubled or trebled by a rise of  $10^{\circ}\text{C}$ . In the realm of plant physiology such a rise has been shown by F. F. Blackman in the case of carbon-assimilation in the leaf. The study of the effect of temperature on the absorption of the hydrogen ion would seem to indicate that this absorption is controlled by some chemical action in the cell, and is not the result of simple diffusion through the plasma-membrane, or of mere adsorption by the cell protoplasm.

That the numbers obtained experimentally show that the rate of the reaction depends merely on the temperature coefficient  $k$ , and the con-

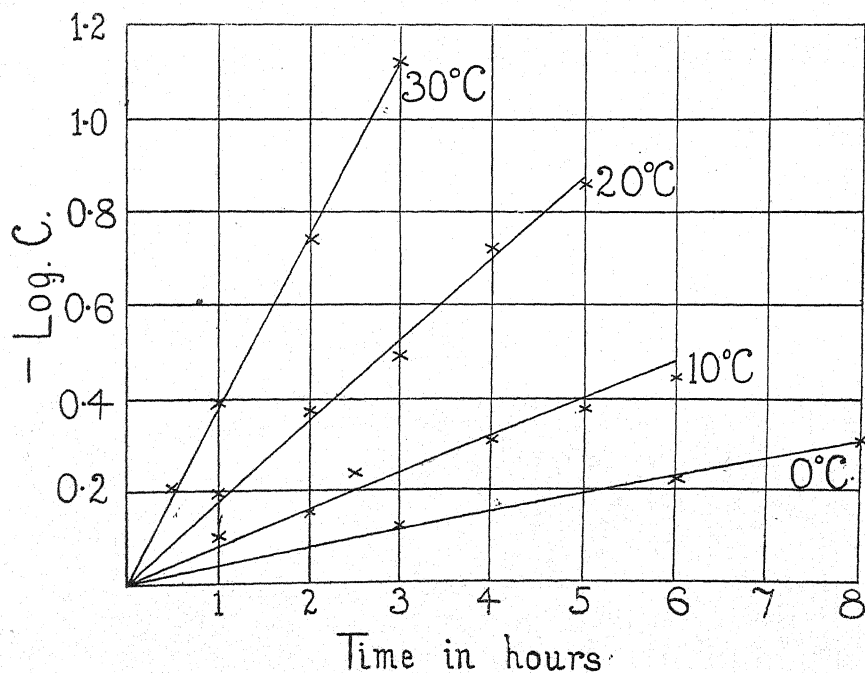


FIG. 4. For explanation see text.

centration of the acid indicates that the quantity of the substance with which the acid reacts, presumably the plasma-membrane, or some part of it, remains constant during the first few hours of the reaction, as it does not influence the rate of reaction. This suggests that either the absorbing substance is present in such large quantity as compared with the acid that the amount changed is small in comparison with the total amount, or that the substance formed as a result of the absorption is broken down again almost as soon as formed. Such a view of the plasma-membrane is held by Pauli and Szűcs who regard the entrance of ions into the cell as due to the reversibility of such a reaction between ions and the plasma-

membrane. We feel, however, that more experimental evidence is required before such theories can be discussed adequately and with profit.

SUMMARY.

1. The absorption of the hydrogen ion of hydrochloric acid in dilute solution by potato cells takes place according to a simple exponential relation between time and the concentration of the acid.

2. The rate of absorption of these ions by potato cells is increased about 2.2 times for a rise of  $10^{\circ}\text{C}$ . between  $0^{\circ}\text{C}$ . and  $30^{\circ}\text{C}$ .

BOTANY DEPARTMENT,  
THE UNIVERSITY, LEEDS,  
*June 14, 1915.*

# The Root-Nodules of the Cycadaceae.

BY

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With Plate XXIX.

THE Cycadaceae are a group of special interest, owing to their retention of a greater number of primitive characters than are possessed by any other living group of Gymnosperms. Representatives of this group probably existed during the Mesozoic Period and were very widely distributed. The living cycads are represented by five eastern genera: *Cycas*, *Encephalartos*, *Stangeria*, *Macrozamia*, and *Bowenia*, and four belonging to the western hemisphere: *Zamia*, *Microcycas*, *Dioon*, and *Ceratozamia*, all of which are tropical or subtropical. In all the genera with the exception of the monotypic genus *Microcycas*, which is confined to Cuba, and has not been examined by the author, there are in addition to the primary tap-root numerous small secondary roots, some of which form the so-called root-nodules.

These structures have been described as 'Coralline roots' in *Cycas*, where they occur abundantly immediately below the surface of the soil and protruding above it. Reinke described them as special organs for aeration, Schneider suggested a symbiotic association, and Life isolated from them an Alga and some Bacteria, and believed them to be concerned in the assimilation of nitrogen as well as aeration. More recently Zach states that they are not symbiotic, but contain a fungal parasite against which the cells react as a phagocyte.

Amongst non-leguminous plants it is now recognized that the Elaeagnaceae, Myricaceae, Podocarpaceae, and the genus *Alnus* have root-nodules, which are definitely concerned with nitrogen assimilation. With these the Cycadaceae must be associated, because Bottomley has isolated from the nodules of *Cycas* not only *Bacillus radiculicola* but also *Azotobacter*, both of which organisms are known to assimilate atmospheric nitrogen. They are, however, of special interest because in their cortex a very definite green ring, the algal zone, is produced by the presence of an *Anabaena*, which has been described by the author.

The first part of the present series of investigations was carried out with material of *Cycas circinalis* and *Encephalartos Hildebrandtii* kindly supplied by the Curator of Chelsea Physic Gardens; and was extended to comprise the genera *Stangeria*, *Macrozamia*, *Zamia*, *Ceratozamia*, *Dioon*, and *Bowenia*, through the kindness of the Director of the Royal Botanic Gardens, Kew, both of whom I desire to thank.

Root-nodules have been found to occur throughout all the cycadean genera, and as in other non-leguminous plants they are perennial modified lateral roots which have diverged from their normal growth owing to their having been infected with the nitrogen-fixing organism *Bacillus radicola*.

In *Cycas circinalis* they are confined to the surface of the soil and the layers immediately below. It has, however, been ascertained that the lower roots frequently become infected with *Bacillus radicola*, which penetrates the root-hairs and enters the cortex, where its presence stimulates the root to become negatively geotropic (Fig. 1), and when near the surface of the soil the tip becomes swollen and subsequently profusely branched, giving rise to a coralloid mass (Fig. 2). Large masses of these structures are visible on the surface of the soil, and they characteristically contain a very definite green ring, the algal zone. The same conditions occur in *C. revoluta* and *C. seemannii*, but some species in cultivation at Kew have not developed these nodules very abundantly.

*Encephalartos Hildebrandtii* and all the species cultivated at Kew exhibit a similar phenomenon, but the individual nodules are larger, and thus, although the branching is very profuse, the clusters are less dense (Fig. 3).

Examination of a large number of nodules from both *Cycas* and *Encephalartos* showed that the nodule tip may become quite large and even branched several times, while no algal zone is present (Fig. 3, *a*, *b*). The Alga therefore lives in the soil, and must at some period gain an entrance to the nodule. The roots which have become negatively geotropic have *Bacillus radicola* in their cortical cells, but they retain their normal appearance. Very soon, however, a ring of structures, apparently lenticels, is formed a little distance from the tip, which subsequently becomes swollen (Figs. 1 and 3 *a*). The lenticels consist of large masses of very loosely arranged parenchymatous cells formed from the phellogen. They soon become infected with *Bacillus radicola* and also *Azotobacter*, the latter at first making their way between the cells, and later entering them (Fig. 9). Gradually in the nodule, i. e. the portion above the ring of lenticels, the outer cells produced by the phellogen increase in size owing to infection with *Bacillus radicola*, and are pushed apart by masses of *Azotobacter*, some of which subsequently penetrate into them, with the result that there is a zone on the outside of the nodule which contains the two nitrogen-fixing organisms associated together (Fig. 9). This zone extends from the basal lenticels

nearly to the meristematic apex. In many cases *Anabaena* filaments are associated with these large loose outer cells, especially in the angles produced by the nodule branching (Fig. 9).

Other nodules have a very definite green algal zone traversing a corresponding area of the nodule, but enclosed by a few layers of cells (Fig. 11). The three organisms *Bacillus radiculicola*, *Azotobacter*, and *Anabaena* now all become concentrated in this area, which consists of a large air space inhabited by the *Anabaena* and *Azotobacter*, and traversed by elongated papillate cells connected with both the inner and outer tissues, thus keeping the zone intact, and being themselves rich in protoplasmic contents, and containing *Bacillus radiculicola* (Fig. 10). The cortical cells between the algal zone and stele are remarkably free from infection, although some contain the two Bacteria, most of them are filled with starch grains or calcium oxalate crystals. Outside the algal zone is a very definite phellogen which produces a few layers of parenchymatous cells towards the zone, and a few very regular cells on the outside. The algal zone always begins very abruptly immediately above a lenticel, but at the apex of the nodule the *Anabaena* appear to make their way gradually between the cells into which also the Bacteria penetrate, thus continuing the growth of the zone with that of the nodule. Sometimes, however, it is localized to quite a small area, perhaps on one side of the nodule. Associated with its production is an extended development of lenticels at some portion of which the zone is always interrupted (Fig. 11). In nodules without an algal zone only a basal ring of lenticels occurs, but where it is present they are produced in connexion with every branch and at quite frequent intervals on the branches themselves (Fig. 3). The nodule often branches dichotomously, but lateral branches also arise, particularly beneath the primary ring of lenticels, which may become nodules or lateral roots (Fig. 5).

Whilst visiting Kew Gardens, one of the *Stangeria Schizodon* plants was observed to have a piece broken from the pot quite near the bottom, and from this hole a number of roots upon which were numerous small nodules were seen to be emerging. This aroused the question whether the nodules in other members of the Cycadaceae were confined to the surface of the soil or not. Their formation here might have been induced by the exposure to light and air resulting from the presence of the hole, or it might be that they were more or less uniformly distributed throughout the pot. On inquiry the latter was found to be the case.

In *Stangeria* small nodules are produced all along the root, each of which branches dichotomously, and thus little clusters are formed (Fig. 4). Infection of the root with *Bacillus radiculicola* occurs through the root-hairs, and these organisms infect the young lateral roots whilst they are traversing the cortex, and thus their growth in length is arrested, and they are stimulated to branch almost immediately they are free from the parent

root. The root-tip may also develop a ring of lenticels and form the beginning of a cluster of nodules. At the base of the nodules which are produced laterally the phellogen becomes very active, and gives rise to a complete ring of lenticellular tissue (Fig. 12). Between this basal ring of tissue and the nodule large groups of *Azotobacter* and sometimes *Anabaena* collect, both of which are also associated with the outer cells of the nodule, as in *Cycas* and *Encephalartos*. As the nodule develops, other lenticellular areas may appear, which are very out-curved with a strong tendency to form a zone of parenchyma parallel with the surface of the nodule, and frequently small localized areas, containing the three organisms so characteristically associated with the outer cells, become completely enclosed (Fig. 13).

Nodules in which an algal zone is developed become very much elongated, always remaining very slender, and branching repeatedly (Fig. 4, *b*). In these nodules the zone occupies a larger proportion of the cortex, and is traversed by many more papillate cells than in *Cycas* and *Encephalartos*. The three symbiotic organisms extend through the outer cortex to the phellogen usually, and are often associated with the outer cells. Nodules have been examined which had enclosed, in addition to an algal zone, certain other areas of Algae and Bacteria on the surface. In branched nodules the two algal zones of respective branches have been found separated from one another by a band of meristematic tissue.

The nodules of *Macrozamia Macleyi* (Fig. 6) and *M. Dennisoni* resemble very closely those of *Encephalartos* in which no algal zone has developed, and no algal zone has been found in these nodules. Correlated with this is the scarcity of nodules on the surface of the soil in the plants at Kew. Their development corresponds with that described above, but the outer cells, in which *Azotobacter* and *Bacillus radicumicola* are found together, are so large and loosely arranged that the surface of the nodule presents an uneven spongy appearance. In some cases the infection with *Azotobacter* extends well into the cortex throughout which *Bacillus radicumicola* is distributed.

In *Ceratosamia mexicana* (Fig. 7), and *Zamia lindenii* (Fig. 5), the nodules observed remain quite small although branched, and are arranged very regularly in two rows along the root. In *Ceratosamia* large numbers are visible on the surface of the soil. A few roots of *Bowenia spectabilis* were also obtained upon which were some very young nodules. In these three genera *Bacillus radicumicola* is present in the cortical cells of the root and nodule. The basal phellogen produces an envelope of parenchymatous tissue as in *Stangeria*, and in some of the older nodules a second phellogen appears in that area inside the old one. The loose outer cells usually have *Azotobacter* associated with them, but no Alga has been found present in these genera.

The roots of *Dioon spinulosum* and *Dioon edule* produce successive



layers of cork which gradually become split and peel off. The cells themselves are rich in tannin. In addition to, and perhaps correlated with, this somewhat extensive development of periderm, large fan-shaped masses of parenchyma are produced at frequent intervals on the roots. There is a greater development of cells resembling lenticellular complementary cells than has been observed elsewhere. Large masses are present at the base of all lateral roots and also nodules (Fig. 14), which are abundant on roots a little below the surface of the soil. The nodules are repeatedly branched; in some cases two rows of small branches are produced from a central portion, but eventually large spherical masses are produced (Fig. 8). These small nodules show the typical structure described above where no algal zone is present. In addition to these, very much enlarged root-tips are visible on the surface of the soil, which also contain *Bacillus radicola* and have a thick periderm in which numerous very large lenticels are developed. One nodule was obtained which had a definite algal zone (Fig. 8, *b*) occupying a large area. Since only those nodules, to which the sun's rays can penetrate, can contain a living green organism, it seems probable that it is in the swollen tips which reach the surface that the *Anabaena* finds its home.

From these investigations the conclusions have been reached that root-nodules are universally present throughout the Cycadaceae, and they are primarily produced by infection with *Bacillus radicola*, and are therefore concerned with nitrogen assimilation. They are adapted to utilize both the nitrogen-fixing organisms *Bacillus radicola* and *Azotobacter* which are known to fix a greater quantity of atmospheric nitrogen per unit of carbohydrate when growing together than separately.

There is also sometimes a fourth symbiont present—the *Anabaena*. It is not, however, essential to the formation of the nodule, since in *Macrozamia*, *Zamia*, *Ceratozamia*, and *Bowenia* it has not been found, and only in one instance in *Dioon*. The presence of lenticels is evidently correlated with the formation of the algal zone, since they are much more abundant on nodules in which it is developed, and the zone itself is always interrupted immediately below some part of the lenticel. These facts, together with the universal occurrence of a ring of lenticels, or a basal ring of parenchyma with an active phellogen, and the presence of *Azotobacter* and *Anabaena* amongst the outermost layers of cells, suggest very strongly that the outer layers of the original nodule become broken down to form the algal zone, and the phellogen of the basal and lenticellular areas is stimulated by the presence of the *Anabaena* to develop a zone of cells, parallel with the surface of the nodule, which gradually extends and forms a protective layer. This is supported by (*a*) the formation of the local enclosed areas on the surface of the nodule, containing the three organisms in *Stangeria*; (*b*) the algal zone retaining a localized position in the nodule which has been observed in *Cycas*, *Encephalartos*, and *Stangeria*, and also

(c) the separation of two algal zones by a meristem which has been observed in *Stangeria*.

The *Anabaena* would naturally confine itself to an area not many cells deep, if photosynthesis were to go on, because of its dependence on light, the intensity of which must be rapidly diminished as it passes through the overlying cells. It is also impossible for the algal zone to be developed in nodules situated far below the surface of the soil, to which the photosynthetic rays of the sun cannot penetrate. In those cases in which the algal zone appears to be entirely absent it may be due to the method of cultivation, possibly to the absence of the specific *Anabaena* from the soil, since nodules are produced so abundantly without it and correspond very closely with those of other genera in which it afterwards develops. The algal zone is an area in which photosynthesis is taking place and probably attracts chemotactically the nitrogen-fixing organisms which require a carbohydrate as their source of energy. The three organisms thus collect together and work symbiotically, undoubtedly benefiting the cycad by the rich supply of elaborated material they produce.

In conclusion my thanks are due to Professor W. B. Bottomley for his sympathy and suggestions during the progress of these investigations.

#### SUMMARY.

1. All the cycadean genera produce root-nodules which are perennial, modified lateral roots, repeatedly branched and typically forming large coralloid masses.
2. They are produced primarily by infection with *Bacillus radiculicola*.
3. A whorl of lenticels or a continuous zone of loosely arranged parenchymatous cells is produced at the base of each nodule.
4. The outer cells always become pushed apart and infected by *Azotobacter* and, if suitable conditions prevail, by *Anabaena* also.
5. The presence of the Alga stimulates the phellogen to produce other lenticels, from which and the basal area a zone of tissue is produced which encloses the original outer cells in which are the Alga and Bacteria.
6. The algal zone is continuous, except immediately below the lenticels, extending from the base nearly to the meristematic apex.
7. The algal zone consists of a large air-space containing *Anabaena* and *Azotobacter* which is kept intact by papillate cells traversing it from both the inner and outer tissues.
8. *Bacillus radiculicola* is chemotactically attracted to the algal zone; thus leaving the cortical cells in which large quantities of starch grains and sphaeraphides are deposited, and in *Dioon*, also tannin.
9. No algal zone has been observed in *Macrozamia*, *Zamia*, *Ceratosamia*, and *Bowenia*, but nodules are produced containing *Bacillus radiculicola* and *Azotobacter*.

10. The Cycadaceae, a group with many primitive characters, are the only nodule-bearing plants known, in which four organisms are associated together symbiotically, viz. two nitrogen-fixing Bacteria, an Alga, and the Cycad.

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# DESCRIPTION OF PLATE XXIX.

*l* = lenticels; *r. t.* = root-tip; *r.* = root; *n.* = nodule; *v. b.* = stele; *g. p.* = meristematic apex; *p.* = phellogen; *f.* = cortex; *b.* = cells containing bacteria; *a.* = loose outer cells; *c.* = periderm; *B. r.* = *Bacillus radicola*; *Az.* = Azotobacter; *An.* = Anabaena; *s.* = starch; *o.* = calcium oxalate.

Fig. 1. Roots of *Cycas circinalis*, some (*n.*) negatively geotropic owing to infection with *Bacillus radicola*. Nat. size.

Fig. 2. Root-nodules of *Cycas circinalis*. Nat. size.

Fig. 3. Root-nodules of *Encephalartos Hildebrandtii*. Nat. size. (*a*) large tip without an algal zone; (*b*) branched nodule without an algal zone; (*c*) cluster with zone developed. Nat. size.

Fig. 4. Root-nodules of *Stangeria Schizodon* (*a*) without algal zone; (*b*) with algal zone. Nat. size.

Fig. 5. Root-nodules of *Zamia Lindenii*. Nat. size.

Fig. 6. Root-nodules of *Macrozamia Macleyi*. Nat. size.

Fig. 7. Root-nodules of *Ceratozamia mexicana*. Nat. size.

Fig. 8. Root-nodules of *Dioon spinulosum* (*a*) spherical masses and large swollen tip; (*b*) with algal zone present. Nat. size.

Fig. 9. Part of longitudinal section of nodule of *Encephalartos* without an algal zone.  $\times 70$ .

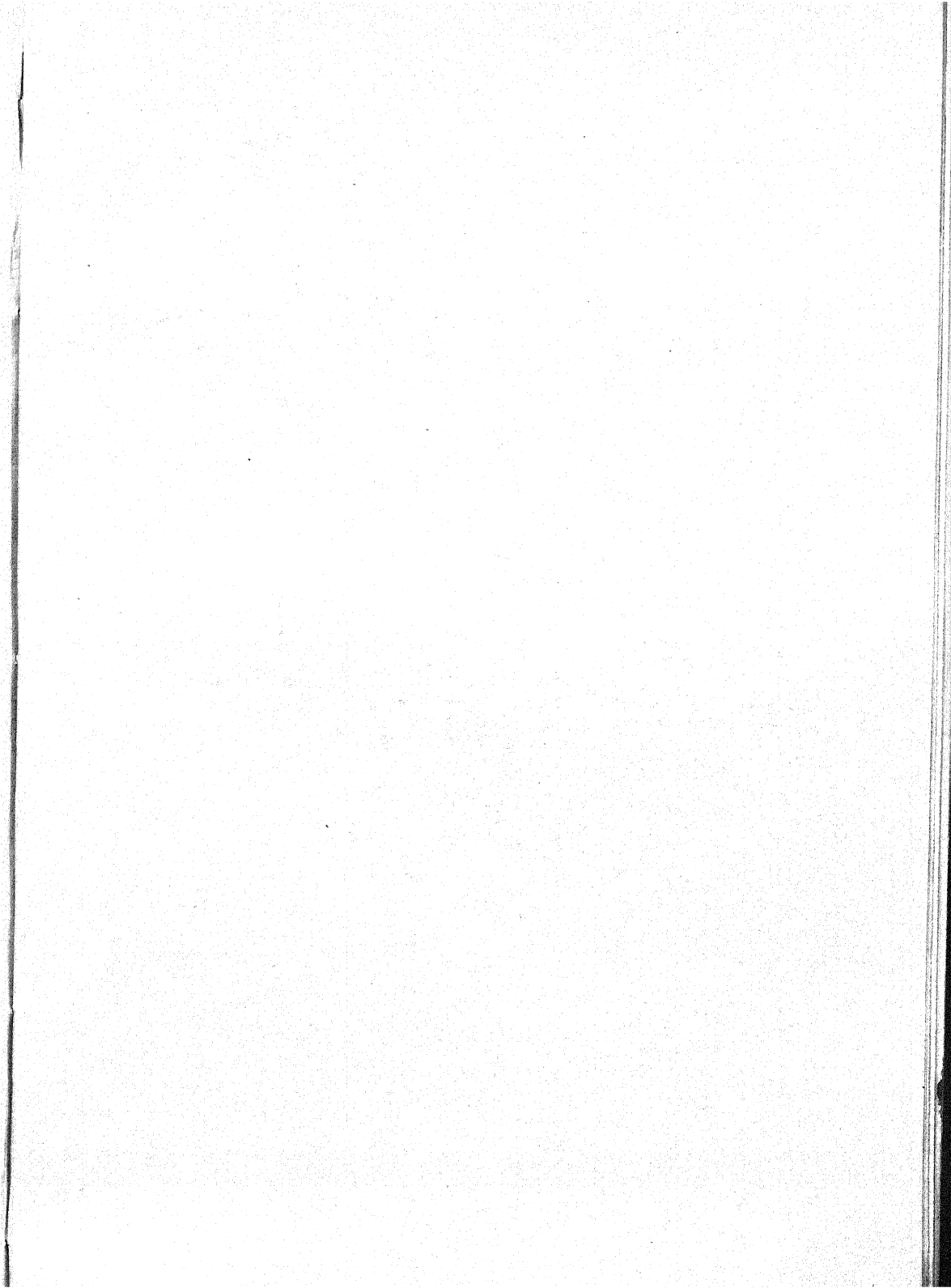
Fig. 10. Portion of the algal zone of the nodule of *Encephalartos*. *p.* = papillate cells. *n.* = nucleus.  $\times 325$ .

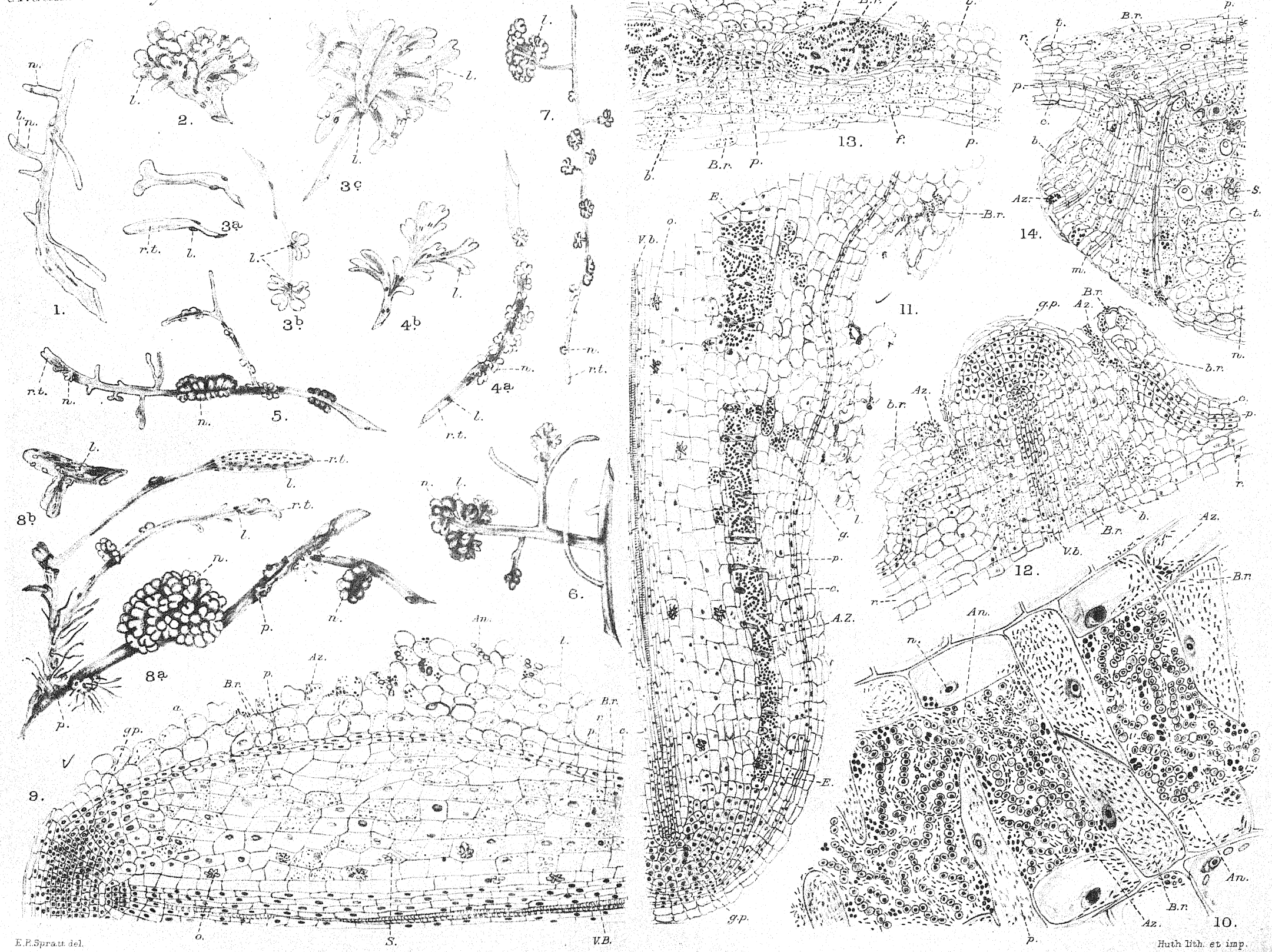
Fig. 11. Part of longitudinal section of nodule of *Encephalartos* with an algal zone. *g.* = gap in algal zone by lenticel; *E.* = end of algal zone. *A.Z.* algal zone.  $\times 35$ .

Fig. 12. Part of longitudinal section of nodule of *Stangeria* showing the basal ridge of tissue = *b.r.*  $\times 35$ .

Fig. 13. Portion of a longitudinal section of nodule of *Stangeria* with enclosed areas of Bacteria and Algae.  $\times 70$ .

Fig. 14. Part of longitudinal section of root and nodule of *Dioon*, showing mass of fan-shaped parenchyma = *b.* at base of nodule; *m.* = meristematic tissue; *t.* = tannin.  $\times 35$ .





E.P. Spratt del.

Huth lith. et imp.

SPRATT—CYCAD ROOT NODULES.





## The Aerating System of *Vicia Faba*.

BY

C. HUNTER, M.Sc.

With six Figures in the Text.

THE exchange of gases which is essential for the respiratory activity of each individual living cell of a green plant is carried on by means of an intricate system of intercellular spaces, which is continuous throughout the plant, and ultimately comes into connexion with the air external to the plant by means of stomata and lenticels. De Bary<sup>1</sup> has carefully described the different forms of intercellular spaces and the manner in which they arise, but the physiological significance of the intercellular spaces of an ordinary green plant cannot be said to have received sufficient emphasis. The aerating system is quite as important to the plant as the water-conducting system or the food-conducting system. In order to obtain some idea of the complete aerating system of a plant, it was determined to work out in detail that of a variety of the Broad Bean—*Vicia Faba*—which is known as the Horse Bean. The plants examined were grown in a mixture of fine soil, sand, and leaf-mould. This potting-soil allowed ready access of air to the roots, and was also rich in food material. The plants were not subjected to any abnormal temperature conditions, as they were grown either in a cold frame or else completely in the open air. The results obtained for each group of plants were similar, and proved that the protection afforded by the cold frame did not affect the structure of the different organs.

The presence of air in intercellular spaces was demonstrated by mounting hand-cut sections either in pure glycerine or in glycerine jelly. Any air which was present in the sections was rendered visible under the microscope, owing to the great difference between the refraction of the rays of light by the gas and by the surrounding medium; and also owing to the reflection of the light rays on striking the under surface of the gas. The air present in the small intercellular spaces of the sections showed up as dark masses, quite distinct from the surrounding cells. This method was particularly successful in the case of longitudinal sections.

<sup>1</sup> De Bary: Comparative Anatomy of Phanerogams and Ferns, p. 201 et seq.

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Seeds of *Vicia Faba* were soaked in water for twelve hours and then sectioned. The structure of the testa is similar to that of *Phaseolus* as described by Haberlandt,<sup>1</sup> and to that of *Pisum sativum* as described by Detmer.<sup>2</sup> The general arrangement of the tissues of the seed-coat is as follows:

(a) An epidermis composed of thick-walled cells of palisade pattern. This layer is compact, and intercellular spaces are completely absent.

(b) A layer of pillar-shaped cells alternating with large intercellular spaces. This form of cells is probably determined, as in water plants, by a demand for increased air space, without interfering with the movement of water from without the seed to those portions which are in need of it:

(c) Tissue consisting of parenchymatous cells having their greatest axis in a direction parallel to the surface of the seed. Intercellular spaces are present in this tissue, but they are of quite a different nature from those of the middle layer, and are quite typical of the intercellular spaces which usually occur in parenchymatous tissue. This layer of cells forms nearly one-half of the soaked testa, but in the dry seed the cells are in a collapsed condition, and only regain their turgid state on the absorption of water.

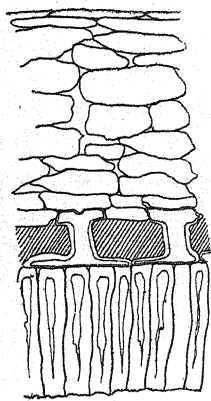


FIG. 1. Testa of *Vicia Faba*, transverse section showing the three types of tissue. The large lacunae of the middle layer are diagonally shaded.  $\times 180$ .

Text-fig. 1 shows the general arrangement of these different layers of cells which go to compose the testa.

Thin hand-sections of the cotyledon were washed gently in water in order to remove the masses of reserve material which would have prevented a careful examination of the tissues. The sections were then mounted in glycerine jelly. The cotyledon was found to consist of three distinct types of tissues:—

(a) An epidermis consisting of small, cubical, compact cells. Stomata were completely absent, and the layer possessed no intercellular spaces.

(b) Vascular tissue devoid of intercellular spaces.

(c) Parenchymatous storage tissue.

The storage tissue is permeated by an intricate aerating system. This does not consist merely of the triangular or rectangular intercellular spaces such as are figured by Sachs,<sup>3</sup> in the case of cells from the cotyledon of *Pisum sativum*. The aerating system of the parenchymatous storage cells of *Vicia Faba* forms a conspicuous feature of the sections, and consists

<sup>1</sup> Haberlandt: Sitzungsber. der K. Akad. Wien, Naturwissenschaften, Bd. 75 (1877), p. 33.

<sup>2</sup> Detmer and Moore: Practical Plant Physiology, p. 192.

<sup>3</sup> Sachs: Lectures on the Physiology of Plants, p. 52.

of continuous intercellular spaces which are capable of bringing about a most active exchange of gases. The gaseous exchange between the living contents of the cells and the air in the intercellular spaces can only take place through the cellulose cell-walls when they are in a moist condition.<sup>1</sup> It has been observed that bands of some material less refringent than air occur in these canal-like intercellular spaces. It seems highly probable that these are masses of water, which assist in the active exchange of gases by keeping the cell-walls in the necessary moist condition. Similar masses have been noted in the intercellular spaces of the cortex in longitudinal sections of the root of *Lupinus*.

The arrangement of the large air-cavities which are present in the stem of *Vicia Faba* is very instructive.

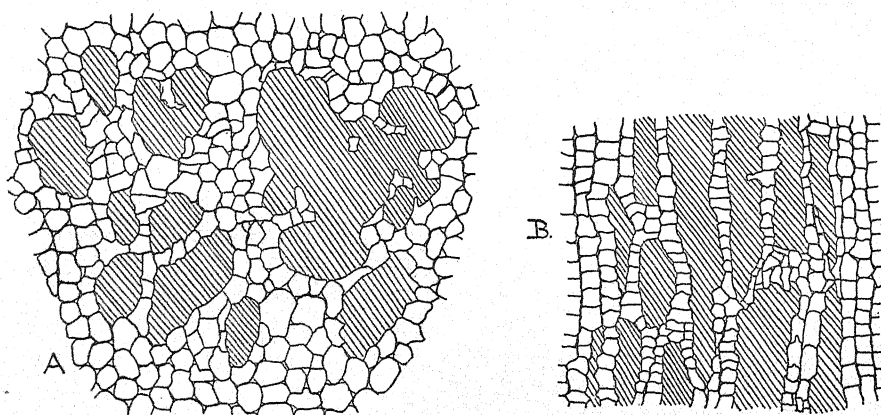


FIG. 2, A and B. Transverse and longitudinal sections of pith of young internodes, showing air-cavities (diagonally shaded) separated by sheets of active cells.  $\times 100$ .

In the youngest internodes the centre of the pith is traversed by strands of active cells separated by well-marked air-cavities, as is shown in Fig. 2, A and B. The structure of the pith in this region is comparable with the arrangement of the ground-tissue in the stems of many aquatic plants. These cavities in the youngest internodes of *Vicia Faba* gradually become smaller as the growing point is approached, until they are reduced to mere chinks in the meristematic cells of the embryonic tissues. It is evident that the cavities are of schizogenic origin, as they result from the unequal development of the plerome in a radial direction. Lower down the stem these strands of active cells disappear, and one large central cavity replaces the much divided cavity of the younger internodes—see Fig. 3. This cavity is produced by the disorganization of the separating strands of cells. The remains of the cell-walls of these strands are to be found along the edges of the cavity. This cavity is therefore produced in

<sup>1</sup> Livingston: The Rôle of Osmotic Pressure and Diffusion in Plants, p. 116.

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a different manner from the smaller cavities above it, and is clearly of lysigenic origin. Here, therefore, is an example of a number of schizogenic intercellular spaces enlarging into air-cavities which are converted into one lysigenic cavity. The transformation might be described in the terms employed by Frank,<sup>1</sup> as of protogenetic origin, because the cavities are really produced in the earliest differentiation of the tissues, and of subsequent hystero-genetic development, since the final form appears only in the older mature stem. In the mature plant this central cavity extends for the greater part of the stem, but in the lower internodes it decreases in size, and ultimately disappears completely. This central air-cavity may be described as a very fine tube, gradually tapering in the oldest internodes and finally disappearing completely, whilst in the region of the stem apex it branches into a number of still finer tubes. It occupies relatively the greatest portion of the area of the cross section of the stem of a young

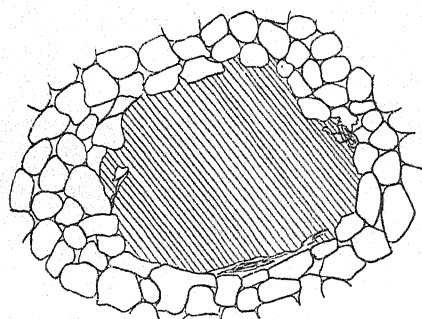


FIG. 3. Transverse section of pith from somewhat older internode than that of Fig. 2. Central cavity developed.  $\times 100$ .

plant in the youngest internodes. In the region shown in Fig. 2, A and B, it occupies about 7 per cent. of the total area of the cross section of the stem. In that part of the stem of a young plant where it forms a fairly wide tube (Fig. 3), it occupies about 4 per cent. of the cross section area. In the lowest internodes it may be reduced to 0.5 per cent., and ultimately dies out. In the full-grown plant the central cavity may occupy 50 per cent. of the cross section of the stem.

The cells at, and near to, the growing point naturally require a large supply of oxygen to assist in the processes necessary for their growth and division. A longitudinal section of the growing point shows that intercellular spaces occur amongst the very youngest cells, and are only absent amongst those cells which have just been formed. It seems quite reasonable to suggest that the large relative area occupied by the cavities in the young internodes is a device to secure a sufficient aerating system for this active region, and that the division of the cavity will assist in the even distribution of the oxygen for respiration, and the removal of the resulting carbon-dioxide.

In the lowest and oldest internodes no central cavity is present, but an interrupted ring of lysigenic cavities, occupying about 2 per cent. of the area of a transverse section, occurs (Fig. 4). It is rather difficult to understand what is the function of this arrangement of cavities. In the

<sup>1</sup> Frank: Beitr. zur Pflanzenphysiologie, p. 101.

exchange of gases which is necessary for root-respiration, the gases must either travel long distances from the aerial breathing organs, or must enter and leave the root by diffusing through the outer walls of the limiting layer of cells. Whatever system is employed must provide for an efficient exchange of gases—the removal of carbon-dioxide, and the provision of oxygen. The following statement by Jost<sup>1</sup> is of interest in this connexion: 'An investigation of the intercellular space system of all plants, whether aerial, subterranean, or submerged, teaches us that the accumulation of carbon-dioxide and deficiency in oxygen never reach a degree worth considering, and hence the means at the disposal of a plant are always sufficient for maintaining a gaseous exchange. Carbon-dioxide to the extent of 5 per cent., and oxygen as low as 8 per cent., are seldom met with in intercellular spaces, and Pfeffer and Celakowski have shown that in the interior of living cells oxygen is never wanting.' Livingston<sup>2</sup> regards the root hair as having the twofold function of absorbing soil water, and acting as a breathing organ.

Wacker<sup>3</sup> is of the opinion that land plants are unable to supply oxygen to their roots by way of their aerial breathing organs. Norris<sup>4</sup> has shown that air passages can be produced in the cortex of roots of *Zea mais*, and that the development of these passages seems to depend upon the quantity of air available in the medium surrounding the root.

Poor aeration resulted in the production of large air passages, but the roots of plants in media where there was a sufficient supply of air had normal intercellular spaces. Investigations by the present writer<sup>5</sup> suggested that artificial soil aeration resulted in increased growth of plants. This has been confirmed by more recent work, and similar results have been obtained in water-culture experiments which have been carried out at the Rothamstead Experimental Station.<sup>6</sup> From this evidence it would appear that the root does obtain some of the oxygen it requires by means of the root-hairs, and that, at any rate in some cases, this must be considerably augmented by a supply from the stem tissues to the living cells of the older portions of the root. To apply this to the

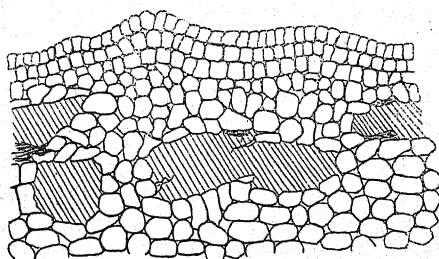


FIG. 4. Cortical cavities in old internodes.  $\times 100$ .

<sup>1</sup> Jost : Plant Physiology, p. 195.

<sup>2</sup> Livingston : *ibid.*, p. 116.

<sup>3</sup> Wacker : Die Beeinflussung des Wachstums der Wurzeln durch das umgebende Medium. Jahrb. wiss. Bot., 32, pp. 71-116.

<sup>4</sup> Norris : Proceedings Bristol Naturalists' Society. Fourth Series, vol. iii, pp. 134-5.

<sup>5</sup> Hunter : Proc. Univ. Durham Phil. Soc., vol. iv, Part 4, p. 186.

<sup>6</sup> Hall, Brenchley, Underwood : Phil. Trans. Royal Soc., B., vol. 209, p. 194.

case of *Vicia Faba*: it will be seen that the cortical air-cavities of the stem would form an excellent means of supplying air to the parenchymatous cells in the cortex of the old root. These cells are a considerable distance from the nearest root-hairs, and the lysigenic cavities in the oldest internodes of the stem may be a device for bringing about a sufficient gaseous exchange in this region.

The intercellular spaces in the ground tissue of the stem are very varied in shape. In young ground tissue they are all triangular in transverse section, but in the mature ground tissue this is not the case. Owing to the fact that the cells vary a great deal in size, the original triangular intercellular spaces give place to more complicated shapes (Fig. 5). These result from the irregular development of the parenchymatous cells, and the subsequent fusing of two or more intercellular spaces. The intercellular space system of the ground tissue of the stem is brought into communi-

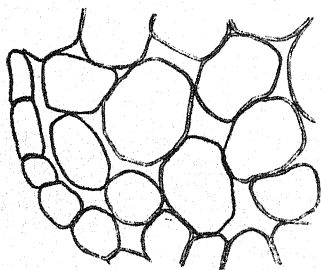


FIG. 5. Ground tissue showing various forms of intercellular spaces resulting from the fusion of two or more spaces.

cation with the external air by means of the stem stomata. The stomatal cavities are small and quite distinct from those of the leaf. In addition to the cavities and intercellular spaces of the stem which have been described, there are present curious cavities one layer of cells below the epidermis. These take the form of long narrow splits, elongated in a plane parallel to the epidermis, and are similar to those which are present in the stem of *Lamium*.

The palisade cells of the leaf of *Vicia Faba* are not arranged regularly. Each palisade cell borders upon an intercellular space. These intercellular spaces are irregular in size—some are small and triangular in a vertical section, whilst others are considerably larger than the cells which they separate. The intercellular spaces of the spongy mesophyll are large and irregular. In a transverse section of the petiole, on the other hand, the intercellular spaces are small and triangular.

A longitudinal section of the root-tip shows that the intercellular spaces are just as important here as at the stem-apex (see Fig. 6, at the bottom). Intercellular spaces are not very marked in the root-cap, but they are present very extensively in the youngest tissues of the root. Probably the air which they contain is obtained from that portion of the root which bears the root hairs. In transverse section these intercellular spaces appear triangular in form. With reference to the older roots, it is worthy of notice that splits often take place in the cortex. How far these are the result of the boring operations of the lateral roots, and to what extent they are due to active growth, has not been determined, but either



primarily or incidentally they take an important part in the respiratory activity of that portion of the root which is some distance from the root-hair bearing region, and also from the aerial portions of the plant.

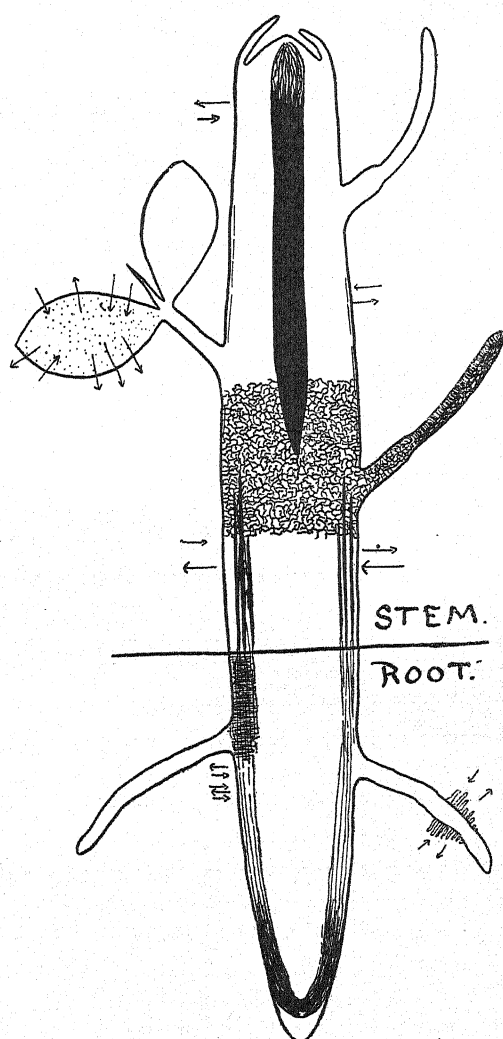


FIG. 6. Schematized longitudinal section of *Vicia Faba* illustrating the distribution of the aerating system in different regions of the plant.

A consideration of the evidence which has been brought together leads to the conclusion that the aerating system of *Vicia Faba* is elaborately adjusted, in order to ensure an efficient gaseous exchange for each living cell, no matter where its position may be in the plant tissues.

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## SUMMARY.

1. The testa of the bean is composed of three definite layers:
  - (a) An epidermis of thick-walled cells.
  - (b) A single layer of pillar-shaped cells.
  - (c) Parenchyma.

Intercellular spaces form a prominent feature of (b), they are present in (c), and absent in (a).

2. The cotyledon of the bean is permeated by a continuous aerating system; stomata are absent.

3. The central air-cavity of the stem of *Vicia Faba* is not uniform in cross section. In the youngest internodes it is much divided, in the central internodes it takes on a circular form and reaches its maximum development, and in lower internodes it is reduced until it finally disappears.

4. The division of the air-cavities in the youngest internodes is probably a means of securing a sufficient gaseous exchange in the active region of the growing point.

5. The small air-cavities of the youngest internodes are of schizogenic origin. The transformation of these into one central air-cavity is of a lysigenic nature.

6. A ring of lysigenic cavities is present in the cortex of the oldest internodes.

7. It is suggested that the production of these cortical air-cavities is a device to assist in the respiration of the cortical cells of the old root.

8. The intercellular spaces in the ground tissue of the stem are originally triangular in cross section. The fusing of two or more of these results in more complex forms.

9. The palisade cells of the leaf are separated by large intercellular spaces.

10. The intercellular space system of the root-tip forms a very important feature of a longitudinal section, and clearly demonstrates that the aerating system is of the utmost importance in the most active regions of cell development.

In conclusion, the author's thanks are due to Dr. Darbishire for the kind interest which he has taken in this work.

THE UNIVERSITY,  
BRISTOL.

# On the Hairs of the Tomentum and Ovary in *Rhododendron Falconeri*, Hook. f., and *Rhododendron Hodgsoni*, Hook. f.

BY

ENID M. JESSON.

With one Figure in the Text.

AS the tomenta of the leaves of *Rhododendron Falconeri*, Hook. f., and *Rhododendron Hodgsoni*, Hook. f. (both belonging to the section *Eu-Rhododendron*), were found to present a different appearance, they were anatomically examined during a critical study of the two species. Further, seeing that the indumentum of the ovary of *R. Falconeri* was also found to exhibit considerable differences in its character and was variously described as 'hirsutissimum viscosum' and 'densely ferruginous woolly', the investigation was extended to that organ. The result of this investigation is embodied in the following paragraphs.

## I. LEAVES.

The leaves of *R. Falconeri* are described by C. B. Clarke in Hook. f. Fl. Brit. Ind. as 'large, long-petioled, elliptic, ferruginous tomentose beneath, very coriaceous', and these, as well as those of the following species, are figured in Hook. f. *Rhododendrons* of the Sikkim Himalaya, tt. xv and x respectively. A leaf of intermediate age was selected for examination, about 18 cm. long and 10 cm. wide. The tomentum itself is particularly conspicuous, forming an orange-brown or cinnamon-coloured covering, soft and velvety in texture, thickest at the midrib and thinning out towards the margin. A portion of this tomentum was scraped off and mounted in glycerine jelly containing gentian violet. On examination it was seen to consist of hairs made up of a delicate network of cells in the form of a funnel, the uppermost cells of which elongate, forming branches or chains of thin-walled cells (Fig. A), the branches being composed of a single row of cells. Frequently individual cells become more or less inflated, approaching a more spherical outline, but as a rule they are elliptic-ovate. The hairs stand at right angles to the surface of the leaf, so that sections cut parallel to it reveal a number of hairs in transverse section, those

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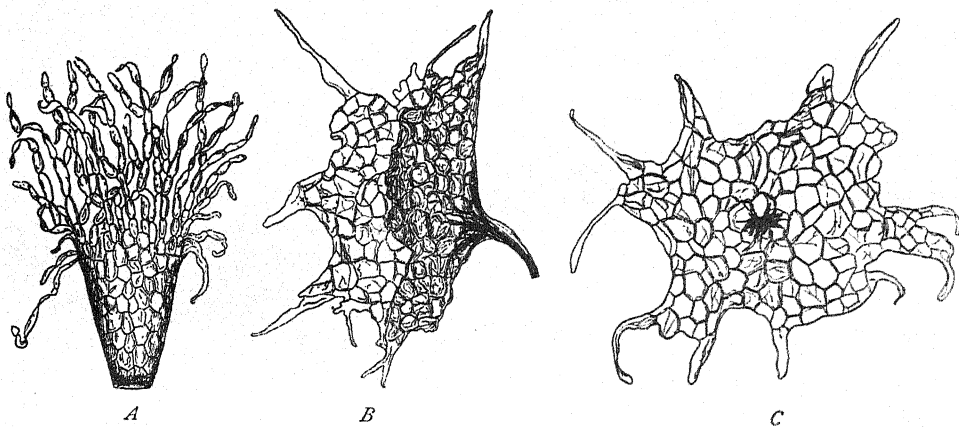
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from the middle and lower part of the hair appearing as continuous rings of collapsed cells, the whole being devoid of contents.

In *R. Hodgsoni* the leaves are described by Clarke, l.c., as 'long-petioled, narrowly obovate-oblong, cinnamomeous or whitish subtomentose beneath'. A medium-sized leaf is about 21 cm. long by 8 cm. broad, but in order to examine the tomentum a young leaf was selected. In this condition the tomentum is of a dull cinnamon colour, granular in appearance and conveying none of the velvety appearance of *R. Falconeri*. In older leaves the tomentum becomes thinner and more scattered, and the individual hairs much smaller, till in the oldest leaves it loses all granular appearance and merely looks like a brown skin. This is not the case in *R. Falconeri*, as the tomentum never loses its felt-like appearance, though in older leaves



Hairs from the tomentum of *Rhododendron Falconeri* (A) and *R. Hodgsoni* (B and C).  
All greatly magnified.

it may become somewhat blackish in colour. The hairs of *R. Hodgsoni* are peltate, and consist of a saucer-shaped mass of cells, having a stalk attached at the centre of the convex surface (Fig. B). Here also, the outermost cells are prolonged, but the arms are much shorter, more pointed, and are usually formed simply by the elongation of the outer cell, without further division, and are thus unicellular and not multicellular as in the previous case. Breitfeld<sup>1</sup> describes a broom-shaped, shaggy hair from the leaves of several species of *Rhododendron*, including those of *Rhododendron Falconeri*. The writer has not been able to find any such hairs on the leaves, but the ovary possesses a very similar type, as will be seen below.

In considering the relation of these trichomes to the other types exhibited by the genus, the typical peltate form seems to be the simplest, and the form from which those now under consideration have sprung

<sup>1</sup> Breitfeld : Der anatomische Bau der Blätter der Rhododendroideae, in Engler's Jahrb. ix (1888), 319, Taf. vi, Figs. 3 and 6.

by further differentiation. Such a primitive peltate hair would consist of a short, multiseriate stalk, the head being composed of an inner circular disc of small cells and an outer annular zone of large elongated cells. This type is of widespread occurrence and is figured by Breitfeld, l. c., from *Rhododendron Malayanum*, Jack. The next stage in the development would seem to be that of *Rhododendron Anthopogon*, Don., where the stalk is longer and the outer zone curved upwards to form a cup, the inner cells, however, are still few in number; a figure of this hair may be seen in Solereder, Systematic Anatomy of the Dicotyledons (English edition), p. 485. From this it is easy to imagine the evolution of the type found in *Rhododendron Hodgsoni* by the extended development of the central disc, and of that in *R. Falconeri* by the upward growth of the main mass of cells, so as to form a funnel rather than a saucer-shaped structure—the stalk at the same time having become reduced (*R. Hodgsoni*) or suppressed (*R. Falconeri*).

## II. OVARIES.

The ovary of *R. Falconeri* was originally described and figured by Sir Joseph Hooker, l. c., tab. x, as 'hirsutissimum viscosum', and the original drawing shows the ovary green and densely covered with short, stiff hairs, with what look like drops of a viscid matter adhering to them. There are no specimens at Kew which can be identified with certainty as being of this collecting on Tonglo. But from plants collected later in the same year (1848) in different localities, the ovary has been found to be not hirsute at all, but apparently glabrous and covered with a copious viscous substance. When examined, this covering was seen to be composed of a large number of glandular hairs embedded in the viscous matter exuded by them. Each hair consists of a very short, multicellular stalk, capped by a large globular head. In specimens from later collections, however, and in many fresh ones, the ovary was covered by a ferruginous felt, and in this case the indumentum consisted largely of fascicled hairs, among which a few of the glandular hairs were interpolated. The former are stellate and shaggy, consisting of a short, multiseriate stalk to which is attached a number of uniseriate branches, pointing in all directions. These are a modification of the broom-shaped shaggy type drawn by Breitfeld, l. c., Fig. 3, in which the arms are very regularly directed upwards. At the same time the branches were scarcely as spreading as shown in Fig. 6 on the same plate (both for *Rhododendron Falconeri*). The present hairs are, in fact, intermediate between the two figures. It is important to note that the glandular and fascicled hairs appear in very varying proportions on the ovary of *R. Falconeri*.

In the case of *Rhododendron Hodgsoni* the ovary possesses the fascicled type of hair only, imparting a whitish appearance and soft texture.

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The above have been treated at some length, not on account of their value as isolated facts, but for the reason that such anatomical details, where constant, are often of the greatest assistance in solving taxonomic problems, especially in the classification of specimens as prone to natural hybridization (even within the limit of one species) as the genus *Rhododendron*. In the case under consideration, the constancy of the character derived from the structure of the hairs seems to be sufficiently established by the examination of a large number of specimens, collected in different localities. Even if the amount of the tomentum varied, the type of hair remained the same for each species.

#### SUMMARY.

1. The orange-brown, velvety tomentum of the leaves of *Rhododendron Falconeri*, Hook. f., is made up of peculiar funnel-shaped hairs, with branches one cell in thickness from the upper portion. That of *R. Hodgsoni* is more scaly, and the individual hairs are saucer-shaped, having a stalk at the centre of the convex surface. The broom-shaped, shaggy hair has not been observed on the leaves of *R. Falconeri*, as described by Breitfeld; but a similar type occurs on the ovary of that species.

2. The tomentum of the ovary of *R. Falconeri* is extremely variable, thus accounting for the discrepancy existing between various descriptions. The ovary is covered by glandular and stellate hairs, these being found in very different proportions—in some cases either one or the other being entirely absent.

3. The types of hair described have been found to be constant in many specimens examined, even if the amount of tomentum varied. It is therefore to be concluded that they are of taxonomic assistance.

My thanks are due to Mr. L. A. Boodle for valuable help during the preparation of this note, and to Dr. O. Stapf, under whose direction the work has been carried out.

## Ernest Lee: 1886-1915.

THE death of Ernest Lee in the trenches of the Western Front on July 10, 1915, has robbed British Botany of a capable teacher and promising investigator, and will be greatly regretted by all who knew him or his work.

He was born on April 11, 1886, at Stanley-Lane End in Yorkshire, whence his family removed to Burnley while he was still a small child. In Burnley, therefore, he grew up, and there, in spite of hard work during the day-time, he contrived to attend the evening classes of the Burnley Technical Institute. He soon developed a deep interest in Natural Science, and so excellent was his work that he obtained a National Scholarship in Geology in 1906. This took him to the then Royal College of Science, London, where his enthusiasm for biology found scope, and where he won a First Class in the A.R.C.Sc. examination in 1909, and in the same year received the Edward Forbes Medal and Prize in Botany and a Marshall Scholarship.

His scholarship enabled him to spend another year in Professor Farmer's laboratories in an investigation, the result of which was his paper on Leaf Fall,<sup>1</sup> a piece of work which brought him into correspondence with various older botanists.

He earned the approval of his fellow students as 'a thoroughly good sort', and one who was always willing to give help. He was highly thought of by the staff and left behind him the reputation of a first-rate worker.

In May, 1910, he was appointed Demonstrator, and in the following autumn Assistant Lecturer in Botany at Birkbeck College, London, where he remained till the autumn of 1913.

During these three years of close association in the work of the department I came to know Mr. Lee well and to think highly both of his character and his abilities. He was one of the keenest colleagues one could have had, always on the track of some scheme for the development of the department, some fresh possibility of research, some method of bringing home to the students the interest of his special subjects.

He worked at various semi-physiological as well as anatomical investi-

<sup>1</sup> The Morphology of Leaf Fall. *Ann. of Bot.*, 1911, p. 51.

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gations, he got under weigh the study of a Mendelian problem, and he published the first of his two papers<sup>1</sup> on seedling anatomy.

He became a Fellow of the Linnean Society and a member of the British Ecological Society.

But it was not only from the professional standpoint that Mr. Lee showed himself eminently likeable. He possessed an enthusiasm which was not merely youthful but based on both experience and reading for all sorts of reform, and especially for such developments as might bring biological considerations within the sphere of politics; and certain discussions—arguments—in which he bore his share are very pleasant memories.

In the autumn of 1912 he was nominated for the chair of Botany in the Ahmedabad Institute of Science, India, and he only did not undertake those distant responsibilities because the medical officer reported him unsuited to the climate. The following autumn he joined the department of Agricultural Botany in the University of Leeds.

Mr. Lee was a good 'shot', and at Leeds he joined the Officers' Training Corps of the University and thus found himself, when war broke out, in a position not only to volunteer but to be of immediate use. He spent August in helping with the organization of the O.T.C., which was thrown open to professional men in Leeds, and he had charge of the musketry. In the beginning of September he obtained a commission as second lieutenant in the 4th Duke of Wellington's (West Riding) Regiment. He became machine-gun officer, and by the end of the month was gazetted lieutenant. Already at the time of his death he had been specially marked for further promotion.

In November, 1914, he married Miss H. S. Chambers, B.Sc., then Lecturer in Botany at the Royal Holloway College, to whom he had been engaged for some two years. It was a marriage which promised all the happiness of shared interests.

On April 12, 1915, Mr. Lee was sent to the front. He was just 29 years of age.

He had hard work; it interested him, and he was happy in it and as full of enthusiasm for his military duties as he had been for his botanical work. His men were devoted to him and his praise of them was high.

His death was caused by a bullet which penetrated the parapet of the trench and went through his head. He was carried down to the dressing-station but was not conscious again, and died within two hours.

It is striking to notice how the impression which Mr. Lee made on his brother officers and on his men coincided with that of his colleagues

<sup>1</sup> Observations on the Seedling Anatomy of certain Sympetalae: I, Tubiflorae. *Ann. of Bot.*, 1912, p. 727. Observations on the Seedling Anatomy of certain Sympetalae: II, Compositae. *Ann. of Bot.*, 1914, p. 303.

and students under very different conditions. To all he was the energetic worker, the interested student, the 'keenest of officers devoted to his work'; and the machine-gunnery of his section who wrote of 'the close friendship between Mr. Lee and his men' will find their echo in the thoughts of his students.

It is in such letters that Mr. Lee's best epitaph may be found :

'He never spared himself when there was work to be done', and 'he died . . . in the execution of his duty'.

H. C. I. GWYNNE-VAUGHAN.